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GUY RICHARD BISBY
1889–1958

T. Johnson

By the death of Guy Richard Bisby, at Staines, England, on September 3, 1958, mycology lost one of its distinguished figures.

Dr. Bisby was born at Brookings, South Dakota, on August 17, 1889. He obtained a B.Sc. degree at South Dakota State College in 1912, an A.M. at Columbia University in 1917, and a Ph.D. at the University of Minnesota in 1918. He held assistantships in 1913–14 at the Brooklyn Botanical Gardens, and 1915–16 at Purdue University, where he worked with Dr. J. C. Arthur on the taxonomy of the rusts. From the time of his association with Dr. Arthur, mycology became the center of his interests. The period 1916–20 was spent at the University of Minnesota, first as instructor in plant pathology and mycology, and later as Assistant Professor. In 1920 he was appointed Professor of Plant Pathology at the Manitoba Agricultural College, Winnipeg.

At Winnipeg he formed an intimate friendship with the noted botanist Professor A. H. R. Buller, which led to their fruitful association in the study of the fungi of Manitoba. This work resulted in a number of publications, of which the most important were *The Fungi of Manitoba*, by Bisby, Buller, and John Dearness, 1929; and *The Fungi of Manitoba and Saskatchewan*, by Bisby, Buller, Dearness, W. P. Fraser, and R. C. Russell, 1938. The work was done with such thoroughness that the authors could truthfully state, in the 1938 publication, that "Manitoba ranks mycologically amongst the better-known areas of the world."

Dr. Bisby made his influence felt in many ways during his stay in Winnipeg—particularly as a member of the Manitoba Natural History Society and through his close connection with the Dominion Rust Research Laboratory. It was through his instruction that several members of its staff became competent mycologists.

While employed in Canada Dr. Bisby made several trips to England, where he made the acquaintance of Dr. E. J. Butler and other noted British mycologists. He was attracted by Kew, the great center of mycological studies, and his association with British workers was already established in 1931, when he published, in collaboration with E. J. Butler, a monograph on the fungi of India. It was through the attraction of the type of work done at Kew that he accepted, in 1936, the post of Assistant Mycologist at the Commonwealth Mycological Institute.

During his years in England he accomplished much of his most important work. He cooperated with T. Petch on *The Fungi of Ceylon*, 1950; with E. W. Mason on a list of British Pyrenomycetes; with Miss E. M. Wakefield on a list of British Hyphomycetes; and with Malcolm Wilson on a list of British rusts.

GUY RICHARD BISBY

The well-known *Dictionary of the Fungi*, produced in collaboration with C. G. Ainsworth, was a product of this period, as was also his book *An Introduction to the Taxonomy and Nomenclature of Fungi*.

His presence on the Special Committee for Fungi of the International Botanical Congress was a recognition of his authority on nomenclature. In 1956 he was given the Outstanding Achievement Award of the University of Minnesota, for "basic contributions to the solution of taxonomic problems (and) the excellence of his mycological services to applied biologists in all parts of the world—"

For a man of his ready wit and acute intelligence, Dr. Bisby was singularly retiring. He never sought honors or deviated far from his chosen path. His unfailing kindness to his students was almost hidden away. To his friends he was a delightful companion, with a vast knowledge of subjects such as gardening and the Stone Age in England, which was one of his hobbies. Another hobby was chess: some of his friends in Winnipeg remember the game of chess he played with Dr. Alekhine when the latter was world's champion. Music was another hobby, and he was an accomplished player on the clarinet. It is sad that the stroke he suffered in 1954 reduced the enjoyment of his last years.

PHYTOPATHOLOGY for May (49: 249-322) was issued May 7, 1959.

INSECT TRANSMISSION OF THE LEAF-CRUMPLE VIRUS OF COTTON[1]

E. F. Laird, Jr., and R. C. Dickson[2]

SUMMARY

Leaf-crumple virus was transmitted from cotton to cotton by the whitefly, *Bemisia tabaci*, but not by *Trialeurodes abutilonea*. It was transmitted by single adults, but immature forms were not able to acquire the virus. Stub cotton plants were as potent virus sources as newly infected plants. Seed transmission was not demonstrated. The acquisition feeding and inoculation feeding thresholds were, respectively, 4–8 and 1–2 hours. The virus was retained by the adult for 5 days and was not transmitted through the egg. Egyptian cotton was also susceptible to the virus. Based on certain virus-vector relationships, the leaf-crumple virus is similar to the leaf-curl virus of cotton, but is distinct from it on the basis of symptoms.

In 1954 Dickson, Johnson, and Laird (2) reported field transmission of the leaf-crumple virus of cotton by grafting and by mixed populations of the whitefly species, *Trialeurodes abutilonea* (Haldeman), and the sweet potato whitefly, *Bemisia inconspicua* (Quaint.), taken from diseased field plants. It next became essential to confirm insect transmission in the laboratory and to determine whether both the species are vectors. In addition, certain virus-vector relationships were studied to evaluate the disease in the field and to provide data for identifying this virus.

MATERIALS AND METHODS.—*Bemisia inconspicua* has been described as a distinct species and has been reported intermingled with *Bemisia tabaci* (Genn.) in South America and in the United States. However, Russell (5) considered it to be a synonym of *B. tabaci*, so the authors now use *B. tabaci* for the insect we previously called *B. inconspicua*. The insects used in this study were from a population found generally infesting cotton and leafy spurge in desert areas of southern California and Arizona.

B. tabaci and *T. abutilonea* were reared separately on healthy upland cotton, *Gossypium hirsutum* Linn., var. *Acala* 4-42. Test insects were confined to plants in large, rectangular cloth cages; cylindrical, cloth sleeve-type cages; or small, plastic leaf cages. The latter were similar in function to those described by Giddings (3), but differed in construction. They were 16 mm in diameter and 9 mm high, and had both ends enclosed with nylon. The end next to the leaf was covered with 50–55-gauge stocking material. An access hole in the side was covered with a paper square held in place by adhesive tape. The cage was secured to the leaf by means of a balsa wood square and a rubber band. With certain precautions, adult whiteflies were transferred in these cages from leaf to leaf. This method largely eliminated the insect mortality caused by excessive handling with the aspiration technique. The leaf cages were placed on the underside of the leaf, the preferred feeding site for these species.

Virus source and test plants were *Gossypium hirsutum* var. *Acala* 4-42 grown in the greenhouse. Age of the test plants at the time of experimental infestation was 9–25 days.

During infestation by the insects, the test plants were held in a greenhouse at 20–30°C. After the test insects were removed, the plants were transferred to a greenhouse room with temperatures of 30–38°C, since symptoms were clearer at the higher temperature. An uninfested plant was left in each pot as a check for greenhouse contamination by viruliferous whiteflies. The greenhouse was fumigated each week, or more often if necessary, with TEPP (tetraethyl pyrophosphate) or nicotine.

VECTOR IDENTITY.—Eight experiments were conducted with pure populations of *B. tabaci* and *T. abutilonea* to confirm insect transmission and to determine their efficiency as vectors of the virus. In each experiment, non-viruliferous adult whiteflies were placed on a leaf-crumple-diseased cotton plant in a large cloth cage for the acquisition feeding period. Then they were removed from the virus source and confined in small, cloth sleeve cages over 16 test plants, 2 plants per cage. Whiteflies that had fed on healthy cotton were placed on 8 test plants and served as checks for viruliferous individuals in the laboratory cultures. Cotton aphids from diseased field plants were also tested as vectors. Table 1 summarizes data from these experiments. *B. tabaci* transmitted the leaf-crumple virus; the other two did not. Under these conditions, *B. tabaci* was able to transmit this virus as efficiently as it does the leaf-curl virus of cotton in Africa (4).

The inability of *T. abutilonea* to transmit leaf-crumple virus is fortunate for the grower, since populations of this whitefly in cotton fields are usually much greater than *B. tabaci* populations.

SINGLE-ADULT TRANSMISSION.—Transmission by single whiteflies was studied with field-collected and laboratory-reared insects. A single adult was removed from a diseased field plant and placed immediately in a sleeve cage over a test plant. In the laboratory, single viruliferous adults in leaf cages that had been given an acquisition feeding period on a diseased cotton plant or reared on diseased cotton were transferred to test plants. Data from these tests are shown in Table 1. Single whiteflies were able to transmit the virus. The failure of transmission by field-collected

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Paper No. 1090, University of California Citrus Experiment Station, Riverside, California.
[2] Laboratory Technician III, and Associate Entomologist.

TABLE 1.—*Transmission of the leaf-crumple virus from cotton to cotton by 2 whitefly and 1 aphid species*

Species	No. insects per plant	Acquisition period (hours)	Inoculation period (hours)	Diseased/test plant nos.	
				Virus source	Healthy source
Bemisia tabaci	25	48	48	50/61	0/30
	25	48	72	7/8	0/3
Trialeurodes abutilonea	25	24	48	0/68	0/32
Aphis gossypii[a]	25		72	0/26	
Bemisia tabaci[a]	1		24	0/97	
	1		72	3/73	
Bemisia tabaci[b]	1	24	72	1/15	
	1	[c]	72	1/40	

[a] Field-collected from diseased plants.
[b] Laboratory reared.
[c] Reared on diseased cotton, length of feeding period unknown.

insects with a 24-hour inoculation feeding period could be attributed to an incomplete latent period in the whitefly. Limited experimental data indicate that the latent period in the vector may be 20 hours or more.

RETENTION.—Five experiments were conducted to determine how long the vector retained the ability to transmit the leaf-crumple virus. In 4 of these tests, whiteflies were collected from diseased field plants and confined in small sleeve cages over test plants for 24 hours. Then the insects were moved to new test plants for 24 hours, and the procedure repeated until there were no live whiteflies to transfer. The single laboratory experiment was conducted in the same way except that the insects were confined in leaf cages and were given a 24-hour feeding period on the virus source before transfer to the test plants. Data from these tests (Table 2) indicate that the virus is of the persistent type. The short life span of the test insects was probably a result of their advanced age and excessive handling during the experiment. However, the level of transmission remained nearly constant during the time they survived, especially for field-collected whiteflies.

ACQUISITION BY IMMATURE FORMS.—Since *B. tabaci* is highly mobile only as an adult, transmission would be expected to be restricted to the mature insect. Nevertheless, the immature, or larval, stage feeds for a considerable time, and acquisition of the virus by this form might be an important factor in the efficiency of the ensuing adults.

Pupae of larvae reared on diseased cotton plants were removed and glued with saliva to the leaves of healthy test plants in a large cage that prevented contamination from whitefly adults other than those that emerged from the pupae. Pupae reared on healthy plants were transferred to test plants and served as culture checks. The emerged adults were allowed to feed until they died. Sixteen plants were infested with 603 pupae from the diseased plant, and about 120 adults emerged. Of 300 pupae from the healthy rearing plant, 77 adults emerged and fed on 6 test plants. None of the plants infested from either source showed disease symptoms. It appears that *B. tabaci* does not become viruliferous by feeding on leaf-crumple-diseased plants as larvae only.

ACQUISITION THRESHOLD.—Results of transmission experiments (Table 1) showed that *B. tabaci* was able to acquire the virus during a 48-hour acquisition feeding period on the virus source plant. Since the percentage of infected plants was high, it was supposed that the acquisition threshold would be less than 48 hours. To determine the threshold, 7 experiments were conducted in which non-viruliferous adults, 20–30 per leaf cage, were given 4 different acquisition feeding intervals. In each experiment, insects fed for a particular interval were placed on a total of 4 test plants for an inoculation feeding period of 72 hours. The acquisition feeding intervals and the number of diseased and test plants were as follows: 1 hour, 0/24; 2 hours, 0/8; 4 hours, 0/20; 8 hours, 3/30; and 48 hours, 50/61. The threshold proved less than 8 hours, but greater than 4.

INFECTION AGE OF VIRUS SOURCE.—Stub, or ratoon, cotton has been considered a primary reservoir of the virus because its presence near a seeded cotton field has been observed associated with a high disease incidence in the field. Stub plants appeared to be readily available to populations of whiteflies early in the growing season before symptoms showed on newly planted seedlings. Nothing was known as to how they compared to newly infected plants as a virus source for the vector.

An experiment, designed on a statistical analysis basis, was conducted to compare plants of long and short infection periods or ages. Two virus source

TABLE 2.—*Retention of the virus during serial transfers by adult whiteflies that had acquisition feeding on field and laboratory virus sources*

Acquisition source	No. insects per plant	Acquisition period (hours)	Diseased/test plant nos. from inoculation feeding during indicated hours after removal from virus source				
			0–24	24–48	48–72	72–96	96–120
Field	25		31/60	31/58	14/32	4/6	
Laboratory	40	24	3/4	0/4	1/4	0/4	1/4

plants were used: one with disease symptoms visible 17 days, and one that had shown symptoms for 2 years before the experiment was started. Non-viruliferous whiteflies were fed at random on these plants for 24 hours and then confined in leaf cages on test plants for 96 hours. There were 12 test plants for each virus source—3 replicates of 4 plants each. The percentages of diseased plants were converted to angle values (angle = arc sin $\sqrt{\text{percentage}}$),[3] and the resulting data subjected to a "t" test for significant difference. The mean angle for the percentage of diseased plants infected by the whitefly with acquisition feeding was 56.0 ± 30.5 on the newly infected source, and 40.0 ± 8.7 on the long-infected source. No significant difference was found between the means. The extremely large standard deviation for the former was in part a result of the large variance between replications. It would have been desirable to increase the number of plants per replicate and the number of replicates for such an experiment. However, the number of available test insects at any one time was restricted by rearing facilities and the rather low reproductive rate of the whitefly in the laboratory. Until a host-range study is completed, stub cotton should be considered the major virus source during the early part of the growing season, and with the same potential as a virus source as newly infected seedlings.

Seed transmission.—The possibility of seed transmission of the virus was also studied. Seeds collected from diseased field cotton plants growing in 3 locations in the Imperial Valley were delinted and planted in a sterilized soil-sand mixture in wooden flats. After the seedlings had emerged, the greenhouse was fumigated weekly to prevent insect contamination. At 40 days the plants were examined for disease symptoms. Of 2130 seedlings examined, none showed symptoms.

Inoculation feeding site.—In an early experiment it was observed that transmission did not occur when viruliferous insects fed only on test plant cotyledons. To test the susceptibility of these plant tissues, 4 experiments were conducted with viruliferous adults that had an inoculation feeding either on true leaves or on cotyledons. Transmission in 15 test plants was 66.6% from feeding on true leaves and 0% from cotyledon feeding. Costa (1) reported similar results when he attempted to transmit abutilon mosaic virus from a *Sida* sp. to cotton plants by *B. tabaci* in leaf cages. An explanation for these failures would be of interest in the study of the phenomenon of virus infection or virus reduplication.

Inoculation feeding threshold.—With the inoculation feeding site known, 6 experiments were conducted to determine the inoculation feeding threshold. Whiteflies in leaf cages were given a 24-hour acquisition feeding on the virus source and transferred to 12 test plants, 4 for each of the inoculation feeding periods. The feeding periods and transmissions obtained were as follows: 1 hour, 0/24; 2 hours, 1/24; and 4 hours, 5/24.

Transovarian transmission.—Transovarian transmission of the aleurodid-borne viruses has not been reported. Since the leaf-crumple virus seemed to be distinct from any of the viruses previously reported on, a study was undertaken of transmission through the vector's egg. The spurge *Euphorbia maculata* L., found to be immune to the virus, was used as the host plant. Viruliferous whiteflies were placed on this plant and allowed time to oviposit, after which they were killed by fumigation. Fifty adults reared from the eggs were placed in leaf cages on each of 8 test cotton plants. No diseased plants were observed.

Transmission to Egyptian cotton.—The Egyptian cotton, *Gossypium barbadense* Linn., var. *Domains Sakel*, is a host of the leaf-curl virus, which is transmitted by *B. tabaci*. Its susceptibility to the leaf-crumple virus was studied to help determine the relationship of the two viruses. Non-viruliferous whiteflies were placed in leaf cages, 50 per cage, for 24 hours on a diseased Acala 4-42 plant. The cages were transferred, 1 per plant, to 6 Domains Sakel test plants. Two of the 6 test plants became diseased, and none of the check plants infested with adults fed on healthy cotton showed symptoms. The virus was recovered by *B. tabaci* from one of the diseased Domains Sakel plants and transmitted back to Acala 4-42. Although the Egyptian Domains Sakel cotton is susceptible to the leaf-crumple virus, the relationship of this virus to the leaf-curl virus is not certain. Symptoms of leaf-crumple disease on Domains Sakel appear to be quite distinct from published descriptions of symptoms of leaf-curl on the same host. It has not been possible to compare the symptoms of these two viruses directly, but they seem distinct, though similar.

Discussion.—Attempts to transmit the whitefly-borne viruses by sap inoculation have been unsuccessful. For the present, comparisons between the viruses must be made on the basis of symptoms, host range (transmission by insect vector), and virus-vector relationships. Although the symptoms differ, both the leaf-crumple and leaf-curl viruses attack Egyptian as well as American cotton. The efficiency of *B. tabaci* in the transmission of the leaf-crumple virus, both by groups and by single adults, approximates that reported by Kirkpatrick (4) for the leaf-curl virus. Results of tests on the acquisition feeding threshold, inoculation feeding threshold, retention by stub cotton, transovarian transmission, and retention of the virus by *B. tabaci* for leaf-crumple virus are similar to those obtained in tests with *B. tabaci* on the leaf-curl virus. The one difference noted so far is that immature forms readily acquire the leaf-curl virus, but not the leaf-crumple virus. The dissimilar techniques used to test for this characteristic may explain the different results. Further investigations will be necessary to clarify the identity of the leaf-crumple virus; however, it seems to be more like the leaf-curl virus than any of the other whitefly-transmitted viruses.

[3] Values taken from tables by C. I. Bliss.

In addition to their usefulness in the identification of the virus, these results of both laboratory and field experiments should prove helpful in evaluating the disease in the field. Such factors as vector population surveys, potential field disease incidence, and control measures should be more easily assessed.

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STREPTOMYCES SPP. AS A CAUSE OF NATURAL FUNGITOXICITY IN SOILS[1]

John L. Lockwood[2]

SUMMARY

Natural loam or muck soils lysed mycelia of several fungi growing on various agar media. Mycelia in cultures killed by propylene oxide were also lysed by natural soils. Destruction of mycelium was usually nearly complete when soil was in contact with cultures for 7–14 days. Germination of conidia was inhibited on agar media that had been covered with sterilized Cellophane and soil for 2–7 days before application of spores. Germ tubes and mycelia from conidia that germinated on such media were frequently inhibited or lysed. Soil sterilized by steam or propylene oxide was without lytic or inhibitory effects. Sterilized soil inoculated with mycolytic isolates of *Streptomyces* spp. and incubated 5–14 days produced lytic and inhibitory effects on test fungi similar to those produced by natural unsterilized soil. The presence of diffusible toxins in soils was indicated by fungitoxic effects when soils and fungus were separated by agar or Cellophane barriers. Diffusible fungitoxic substances produced by *Streptomyces* spp. are thought to play a role in natural soil fungitoxicity.

Inimical effects of natural soils on fungus spores and mycelium have been described (3, 4, 5, 6, 7, 10), and the concept of a general, widespread fungitoxicity in soils has recently been developed (4, 5). The cause and nature of the fungitoxic property of soil is as yet poorly understood, but evidence indicates the presence of diffusible toxic substances in natural soils (6, 10). Actinomycetes contribute to the fungitoxic property of soil, as shown by Stevenson (9), who found that sterilized soils inoculated with actinomycetes antagonistic to *Helminthosporium sativum* Pam., King & Bakke inhibited germination of conidia and lysed mycelium of this fungus. The present report describes toxic effects of natural soils and sterilized soils inoculated with mycolytic isolates of *Streptomyces* spp. on several fungi, with particular emphasis on lysis of fungus mycelium.

Materials and methods.—Mycolytic isolates of *Streptomyces* spp. were obtained by the method of Carter and Lockwood (2). Suspensions of soil in water were sprayed on 4-day-old cultures of *Glomerella cingulata* (Ston.) Spauld. & Schrenk on peptone agar (2). Colonies that lysed mycelium of this fungus were transferred to yeast-extract–glucose agar (2).

To determine the toxic effects of soils on conidial germination and on mycelium, the following plant-pathogenic fungi were used as test organisms: *Aphanomyces euteiches* Drechs.; *Colletotrichum circinans* (Berk.) Vogl.; *Fusarium oxysporum* Schlect. f. *pisi* (Linford) Snyd. & Hans., race 1; *Fusarium solani* (Mart.) App. & Wr. f. *pisi* (F. R. Jones) Snyd. & Hans.; *G. cingulata*; *H. sativum*; *Rhizoctonia solani* Kühn; and *Verticillium albo-atrum* Reinke & Berth. Fungi were cultured on PDA (potato-dextrose agar) (2). For seeding agar plates for lysis tests, about 2,500,000 conidia in 50 ml of water were added to 250 ml of melted agar at 42°C. Cultures of *A. euteiches* and *R. solani* were prepared with mycelial inoculum. Media used were peptone agar, PDA, and Czapek's agar (per liter: $NaNO_3$, 2 g; K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; sucrose, 30 g; agar, 20 g).

For determining lysis of mycelium, Petri dishes containing 15 ml of seeded agar were incubated 4 days, after which the culture was covered with soil. After 7–20 days, soil was washed from the surface of the agar and cultures were examined under a microscope using a 43× objective. Counts were made of the number of hyphal strands crossing the periphery of the field. Usually, 5 fields were counted on each of duplicate plates. In an alternative method, pieces of agar culture about 4 mm square were crushed between 2 glass slides. The amount of mycelium as compared with untreated controls was estimated using a microscope with a 10× objective. Ratings were

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[2] The writer expresses his appreciation to Mrs. Irene Brock and Mrs. Mary J. Bandurski for technical assistance.

TABLE 1.—*Effect of unsterilized or sterilized loam soil on living or killed mycelia of several fungi on peptone agar when cultures were covered 20 days*[a]

Fungus	Number of hyphae intersecting periphery of microscope field[b]					
	Living cultures			Killed cultures		
	Control	Sterilized soil	Unsterilized soil	Control	Sterilized soil	Unsterilized soil
Colletotrichum circinans	26	23	0	25	27	2
Fusarium oxysporum f. *pisi*	81	80	0	89	80	0
Fusarium solani f. *pisi*	27	27	0	26	30	32
Glomerella cingulata	70	72	0	71	71	1
Rhizoctonia solani	22	22	1	22	23	0
Verticillium albo-atrum	34	36	1	36	36	28

[a] Mycelium was killed and soil was sterilized by propylene oxide.
[b] Average number of hyphae counted in 5 fields on each of 2 plates using a 43× objective.

0–4: 0, no mycelium; 1, 1–10%; 2, 11–50%; 3, 51–99%; and 4, no mycelium lysed. Two squares were rated from each of duplicate plates. Ratings of squares from the same plate or from duplicate plates rarely differed.

Several methods were evaluated for determining the inhibitory effect of soils on germination of fungus conidia on 2% agar: a) Glass slides coated with agar containing conidia were buried in soil (3). b) Conidia were streaked on agar in Petri dishes, which were then covered with soil. c) Soil was placed on agar in Petri dishes for 5–12 days and then washed off, and conidia were streaked on the agar. d) This method was similar to "c" except that sterilized Cellophane was interposed between soil and agar. e) A layer of 2% agar in a Petri dish was covered with a layer of 1.5% agar, which was covered with soil. After 5–12 days, soil and top agar layer were removed and conidia were streaked on the bottom layer. f) This method was similar to "e" except that conidia were streaked on the bottom layer before the top layer was poured. Germination counts were made about 24 hours after conidia were applied.

All methods demonstrated inhibition of germination of conidia by natural unsterilized soil. Methods in which conidia were in direct contact with soil were least satisfactory, because of difficulty in removing soil without damaging agar or removing spores. The Cellophane (d) and double agar layer (e) methods gave clean and apparently sterile agar surfaces, and were used in this study. When natural soil was used, it was necessary to remove the Cellophane within 10 days to 2 weeks, because the film tended to decompose after this period.

Soils used were Conover loam and muck, both collected at East Lansing, Michigan. For inoculation with isolates of *Streptomyces* spp., screened soil was moistened to a point short of stickiness and placed in 250-ml flasks to a depth of 1 in. Flasks of soil were sterilized by autoclaving 1 hour on each of 3–4 successive days, or by exposure to propylene oxide fumes in a desiccator for 3–4 days. Sterility tests were made by plating samples on yeast-extract–glucose agar. Soils were inoculated by adding 2 ml of a suspension of spores of a Streptomyces isolate to each flask. Following inoculation, flasks were sealed with aluminum foil to retard drying of the soil. Flasks were incubated 7–20 days before use.

RESULTS.—*Lysis of fungus mycelium.*—In preliminary tests to determine the effects of soil on fungus mycelium, cultures of *G. cingulata* growing on peptone agar in Petri dishes were buried 14 days without lids 4 in. deep in loam soil in the field. When the plates were retrieved, washed, and examined under a microscope, nearly all the mycelium had disappeared.

Lysis of fungal mycelium was compared in cultures buried in soil in the field and cultures covered with soil in the laboratory. Peptone agar cultures of *A. euteiches*, *C. circinans*, *F. oxysporum* f. *pisi*, *F. solani* f. *pisi*, *G. cingulata*, and *V. albo-atrum* were used. In both tests, mycelia of all fungi except *R. solani* had lysed partially or completely in 14 days. Since results in field and laboratory were similar, all later work was done in the laboratory.

Quantitative estimates were made of the lysis of fungus mycelium by soils. Living and killed cultures of the fungi described above, on peptone agar, were covered with loam or muck soil. Soil was washed off and mycelia counted after 9 and 20 days (Table 1). At 9 days, both soils had caused extensive lysis of living and killed mycelium of all fungi except killed cultures of *F. solani* f. *pisi* and *V. albo-atrum*. At 20 days, with the same exceptions, practically all the mycelia in these cultures had disappeared. Stromata of *C. circinans* and microsclerotia of *V. albo-atrum* resisted lysis. Conidia of *G. cingulata*, *V. albo-atrum*, and *C. circinans*, and conidia and chlamydospores of the *Fusarium* spp. were abundant on the surface of the agar after lysis of the mycelium. Sterilized soil had no effect on either living or killed mycelium. Results were similar when the experiment was repeated.

The germination was tested of conidia from lysed cultures of *C. circinans*, *F. solani* f. *pisi*, *G. cingulata*, and *V. albo-atrum*. Cultures were mixed with water in a blender, and centrifuged 15 minutes at 20,000 rpm, and conidia were collected from the supernatant liquid. In germination tests their viability, as compared with that of spores from PDA cultures, was not impaired.

Two experiments were made to determine whether mycelia in media other than peptone agar were sus-

TABLE 2.—*Effect of sterilized loam soil inoculated with isolates of Streptomyces spp. and incubated 14 days on living or killed mycelium of 3 fungi when cultures were covered 10 days*[a]

Streptomyces isolate	Number of hyphae intersecting periphery of microscope field[b]					
	Living cultures			Killed cultures		
	Glomerella cingulata	*Verticillium albo-atrum*	*Fusarium solani*	*Glomerella cingulata*	*Verticillium albo-atrum*	*Fusarium solani*
10	8	0	23	35	20	22
53	3	2	24	32	17	26
54	0	6	23	32	18	24
Unsterilized soil	0	3	2	0	1	9
Sterilized soil	31	24	28	33	20	25
Control	36	21	25	34	22	29

[a] Mycelium was killed and soil was sterilized by propylene oxide.
[b] Average number of hyphae in 5 fields on each of 2 plates using a 43× objective.

ceptible to lysis by natural soils. Cultures of *F. solani* f. *pisi*, *G. cingulata*, and *V. albo-atrum* growing on peptone agar, PDA, or Czapek's agar were tested. The mycelium, both aerial and submerged, was much more dense on the richer media than on peptone agar. Nevertheless, destruction of living and killed mycelium of all 3 fungi was virtually complete in 3 weeks on peptone agar and Czapek's agar. Only traces remained of mycelium in living cultures of *F. solani* f. *pisi* and killed cultures of all 3 fungi on PDA. It is evident that lysis is not limited to mycelium growing on media deficient in nutrients.

The diffusible nature of the lytic substances in natural soil was indicated by lysis of mycelium within the agar as well as on the surface. In experiments directed toward this point, cultures of *G. cingulata* were covered a) with a layer of 2% agar, b) with sterilized Cellophane, and c) with agar plus Cellophane. Mycelia of cultures so treated and covered with soil lysed in all cases.

Sterilized soils inoculated with mycolytic isolates of *Streptomyces* spp. were tested for lytic effects on mycelia of fungi growing on peptone agar. Isolates 10, 53, and 54 were introduced into sterilized loam soil and incubated 14 days, after which soils were placed on peptone agar cultures of *F. solani* f. *pisi*, *G. cingulata*, or *V. albo-atrum*. Results of 2 experiments were similar (Table 2). Soils inoculated with each of the isolates of *Streptomyces* spp. almost completely destroyed living mycelium of *G. cingulata* and *V. albo-atrum* in 10 days, but had little or no effect on *F. solani* f. *pisi*. Killed mycelium was not lysed by inoculated soils in these tests. Unsterilized soil caused almost complete disappearance of both living and killed mycelia of all 3 test fungi, except for killed mycelium of *F. solani* f. *pisi*, of which about a third of the hyphae remained. Sterilized soil did not affect mycelium.

Loam soil inoculated with mycolytic isolates 2, 25, 28, and 66 of *Streptomyces* spp. was similarly tested in 3 experiments for lysis of mycelium of *F. solani* f. *pisi*, *G. cingulata*, *H. sativum*, and *V. albo-atrum*. Inoculated soils were incubated 18 days, and then placed on fungus cultures for 14 more days (Table 3). Natural, unsterilized soil nearly completely destroyed living and killed mycelia of all 4 test fungi. Soils inoculated with isolates of *Streptomyces* spp. or a mixture of the 4 isolates were somewhat less lytic than natural soil, in general, but in all instances mycelium was at least partially destroyed. In all 3 experiments, killed mycelium was lysed more readily than was living mycelium, the reverse of the situation with isolates 10, 53, and 54. Quantitatively, however, the results of the 3 tests, with respect to soils inoculated with individual isolates, were inconsistent. It is evident that soils inoculated with mycolytic *Streptomyces* isolates exert on living and killed fungus mycelia a lytic effect similar to that of natural soil.

Inhibition of germination of conidia.—The effect of natural unsterilized loam soil on germination of

TABLE 3.—*Effect of sterilized loam soil inoculated with isolates of Streptomyces spp. and incubated 14 days on living or killed mycelium of 4 fungi when cultures were covered 17 days*[a]

Streptomyces isolate	Lysis rating[b]							
	Living cultures				Killed cultures			
	G. cingulata	*V. albo-atrum*	*H. sativum*	*F. solani*	*G. cingulata*	*V. albo-atrum*	*H. sativum*	*F. solani*
2	0	2	1	3	2	0	2	0
25	1	0	2	2	2	0	2	0
28	0	1	1	2	0	0	1	0
66	0	2	1	1	0	0	1	0
Mixture	0	2	0	2	2	0	1	0
Unsterilized soil	0	1	0	1	0	0	0	0
Sterilized soil	4	4	4	4	4	4	4	4
Control	4	4	4	4	4	4	4	4

[a] Cultures were killed by propylene oxide fumes. Soil was sterilized by autoclaving.
[b] Average rating of 2 slide mounts from each of 2 Petri dishes. 0 = no mycelium; 1 = 1–10%; 2 = 11–50%; 3 = 51–99%; 4 = no mycelium lysed.

TABLE 4.—*Effect of sterilized loam soil inoculated with isolates of Streptomyces spp. on germination of conidia of 3 fungi*[a]

Streptomyces isolate	Average % germination of conidia[b]		
	Glomerella cingulata	*Verticillium albo-atrum*	*Fusarium solani*
10	15	12	55
28	6	11	59
66	57	23	59
Mixture	2	0	53
Unsterilized soil	4	0	54
Sterilized soil	95	77	56
Control	94	79	59

[a] Soil cultures were incubated 8 days, then added to double agar-layer plates. After 6 days, soil and the top agar layer were removed, and spores were streaked on the bottom layer.

[b] Average per cent germination based on examination of 10 fields on each of 2 plates with 30–60 conidia per field.

fungus conidia was tested in 8 experiments using the Cellophane or double-agar-layer technique. Germination of *G. cingulata* conidia on agar covered with natural soil was 0–55%—10 per cent or less in 5 tests. Germination of *V. albo-atrum* conidia was 0–18%, and was prevented in 5 experiments. Only 6% of *F. solani* f. *pisi* conidia germinated in one experiment, but germination was not affected in 7 other tests. In 2 experiments with *H. sativum*, germination was 25 and 58%. Muck soil reduced germination of conidia of *G. cingulata* to 10%. Germination of *F. solani* f. *pisi* conidia in controls was 55–65%; germination of conidia of other fungi in controls almost always exceeded 90%. Germination of conidia treated with sterilized soils never differed from that of controls.

Sterilized soil inoculated with mycolytic isolates of *Streptomyces* spp. was tested for inhibition of conidial germination. Inhibition was inconsistent. Germination of conidia of *G. cingulata* and *V. albo-atrum* was less than 50% in 3 of 6 tests, and germination of *F. solani* f. *pisi* conidia was prevented in one experiment. Soil inoculated with isolate 10 consistently inhibited germination of conidia, whereas soil inoculated with isolates 2 or 25 did not affect conidia of any test fungus in 2 tests. Soils inoculated with other isolates were inhibitory in some tests, and not in others. Table 4 shows typical results from an experiment in which inhibition occurred. Mixtures of isolates were seldom more inhibitory than the best of single isolates. In one experiment, however, soil inoculated with a mixture of isolates 10, 28, and 66 gave results closely approximating those with natural soil (Table 4).

The longer soil was in contact with the Cellophane or top agar layer, the greater was the inhibition of germination of fungus conidia (Table 5). For example, germination of conidia of *G. cingulata* was 99% when soil inoculated with isolate 10 was in contact with Cellophane for 4 days. After 9 days, germination was reduced to 25%; after 12 days, to 16%; and after 16 days to 9%. Results were similar with conidia of *V. albo-atrum* and with soils inoculated with isolate 54. Some of the inconsistency of results in germination tests might be explained by too brief a contact between soils and test surface.

Although the inhibitory effects of inoculated soils on conidial germination were usually not as striking as were lytic effects, it was frequently observed that germ tubes of conidia on agar covered with natural or inoculated soils ceased growth and frequently lysed, whereas in controls germ tubes grew into dense mycelium.

The effect of replacement of soil on germinating conidia was studied with the double-agar-layer method. Conidia of *G. cingulata* and *F. solani* f. *pisi* germinated normally on agar that had been covered with unsterilized loam or muck soil for 2 days; conidia of *V. albo-atrum* were inhibited slightly by loam soil. Soil was replaced directly on the cultures after counts were made. Two days later, lysis of mycelium of all 3 fungi was nearly complete.

In a similar experiment, 10-day-old soil cultures of *Streptomyces* isolates 2, 25, 28, and 66 were replaced on conidia following germination counts. There was little inhibition of conidia of *F. solani* f. *pisi*, *G. cingulata*, *H. sativum*, or *V. albo-atrum* by natural loam soil, and none by soils inoculated with the 4 *Streptomyces* isolates at 6 days. After 7 more days, no mycelium remained in cultures treated with natural soil, and only traces (rating of 1) remained on plates treated with soils inoculated with isolates 25, 28, and 66, except for *F. solani* f. *pisi*, which was rated "2" for all isolates. Mycelia of all 4 fungi treated with soil inoculated with isolate 2 also were rated "2."

The results of the inhibition experiments support

TABLE 5.—*Effect of sterilized loam soil inoculated with isolates of Streptomyces spp. on germination of conidia of Glomerella cingulata and Verticillium albo-atrum when agar medium was covered for various periods*[a]

Days	Average % germination of conidia[b]					
	Isolate 10		Isolate 54		Sterilized soil	
	G. cingulata	*V. albo-atrum*	*G. cingulata*	*V. albo-atrum*	*G. cingulata*	*V. albo-atrum*
4	99	95	92	98	100	100
9	25	62	94	87	95	99
12	16	7	97	11	94	98
16	9	13	1	6	98	93

[a] Soil cultures incubated 18 days, then added to plates of water agar covered with sterile Cellophane. At various intervals, Cellophane and soil were removed, and spores were streaked on agar.

[b] Average per cent germination based on examination of 10 fields on each of 2 plates with 30–60 conidia per field.

those of the lysis tests in indicating the presence of diffusible fungitoxic substances in natural soils and suggesting a causal role for antagonistic isolates of *Streptomyces* spp. in natural soil fungitoxicity.

DISCUSSION.—A natural soil fungitoxicity possibly similar to that described from other parts of the world exists in soils in Michigan. The possible role of antibiotics in natural soil fungitoxicity is suggested by the work of Stevenson (9), who found that specific morphological effects on *H. sativum* produced by actinomycete antibiotics *in vitro* were similar to those produced by soils inoculated with the corresponding actinomycete. Despite frequent failures to demonstrate their presence in soils by extraction techniques, a balance between antibiotic production and inactivation could result in the continual presence of small but biologically active quantities of antibiotics in soils. Factors other than antibiotics may also be involved in soil fungitoxicity. Antibiotics lysed living mycelium but not killed mycelium in culture (1), whereas natural soils, certain Streptomyces isolates, and soils inoculated with lytic isolates of *Streptomyces* spp. lysed killed, as well as living, mycelium. Other substances, perhaps enzymes produced by actinomycetes, may be involved in the lysis of killed mycelium, and perhaps living mycelium as well. Since the destruction of mycelium by isolates of *Streptomyces* spp. in mixed culture with fungi was seldom as complete as by natural soils or sterilized soils inoculated with *Streptomyces* isolates, potentiating factors in soil may also be involved (9).

The role of microorganisms other than actinomycetes in natural soil fungitoxicity is yet to be elucidated. The destruction of mycelium of *Fusarium roseum* (Lk.) emend Snyd. & Hans. by sterilized soil inoculated with *Bacillus macerans* Schardinger (8) suggests that actinomycetes are not unique in this respect.

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INHERITANCE IN COWPEA OF RESISTANCE TO TOBACCO RINGSPOT VIRUS[1]

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SUMMARY

Inheritance in Black cowpea of resistance to local lesioning by tobacco ringspot virus was studied in F_1, F_2, and backcross generations. A nearly perfect fit for inheritance of a single gene dominant for resistance was found. The appearance of the F_1 resistant reaction was generally distinct, but no precise separation from the resistant parent type could be made in every case in the F_2. The source of resistance in the material used was unknown, but appears to have originated with a single heterozygous individual. The resistant reaction is an expression of hypersensitivity rather than immunity; the susceptible reaction is expressed by large local lesions, systemic invasion, top necrosis, and death of the plant.

A small quantity of Black cowpea seed, originally from the W. R. Robinson Seed Company, Cairo, Georgia, was obtained in 1955 for local-lesion testing of cucumber mosaic virus. The material was used during the following winter, with increasingly erratic results. Preinoculation darkening of the plants (4) did not increase production of lesions enough for quantitative work with our virus isolates. To obtain reliable test material 16 plants displaying typical cucumber mosaic lesions were grown to maturity, and the seed was saved.

During the early fall months the reaction of this stock to cucumber mosaic was generally satisfactory. Inoculations in late winter, however, gave markedly fewer lesions, possibly because of increased light intensity (1). On testing isolates of tobacco ringspot from muskmelon and cucumber, however, it was found that about ¼ of the seedlings were susceptible, displaying the well-known type symptoms: large local lesions (2, 3), followed by systemic invasion and ultimate death of the plant from top or stem necrosis. The remaining plants displayed varying numbers of small chocolate-brown lesions resembling cucumber mosaic lesions, and almost no systemic involvement. The majority of these were able to bear fruit, even though inoculated at several different stages of growth. Tests on the progeny of these resistant plants showed them all resistant to tobacco ringspot.

MATERIALS AND METHODS.—Individual plants were indexed for resistance and susceptibility to tobacco ringspot virus, and appropriate crosses were made with them to determine the genetic basis of the resistant reaction. Most of the indexing inoculations were made with sap dilutions of 1:30 or 1:50 in 0.01*M* or 0.1*M* phosphate buffer at pH 7.0. Inoculations were made with cotton-tipped swabs dipped in an inoculum-carborundum mixture and rubbed onto carborundum-dusted primary leaves. Most plants at inoculation were 11–13 days old, with the primary leaves well expanded and the trifoliate leaves not yet emerging. Inoculation procedures generally followed those described as successful with this virus (2, 7). Plants were grown at about 75°F on open greenhouse benches, in full light without supplemental illumination. An isolate from muskmelon of tobacco ringspot that resembles Price's No. 1 in symptoms on cowpea and *Nicotiana* spp. (3) was used throughout the trials. Comparative inoculations, made occasionally with isolates from watermelon (G. S. Pound, University of Wisconsin), tobacco (R. W. Fulton, University of Wisconsin), tulip (W. J. Hooker, M. S. U.), and cucumber, always produced very similar results (Fig. 1-A). All plants used in the inheritance studies were individually indexed and classified as to resistance or susceptibility.

To obtain P_1, P_2, and F_2 seed for testing, parent and F_1 plants were allowed to self-pollinate. F_1 and backcross seeds were obtained by transferring appropriate pollen to the stigma of hand-emasculated flowers. Pollinations were begun about 11 weeks after inoculation, and seeds matured about 4 weeks later. Fifteen plants that appeared highly resistant to tobacco ringspot were grown in the greenhouse and used for resistant parent material. A group of susceptible parent plants were saved and grown to maturity by removing inoculated primary leaves as soon as reliable symptoms appeared and before the virus had become systemic. Seeds of the resistant and susceptible parents and the F_1 generation were obtained in the spring of 1957. Selfs of the F_1 lines (F_2 seeds) were obtained in the field during the summer of 1957, and various backcrosses of F_1 to parental lines were made during the winter of 1957–1958. Test inoculations for reaction of the various progenies to tobacco ringspot were begun in October of 1957 and completed in April, 1958.

EXPERIMENTAL RESULTS.—As shown in Table 1, the entire group of plants from lines previously classified as susceptible produced 485 susceptible individuals and 0 resistant. The resistant lines all proved homozygous for resistance, with 350 resistant and 0 susceptible. The F_1 ratio from 26 combinations was 135 resistant to 0 susceptible. The F_2 ratio, based on field selfs of 14 F_1 progenies, was 600 resistant to 195 susceptible. Backcrosses to the susceptible parents

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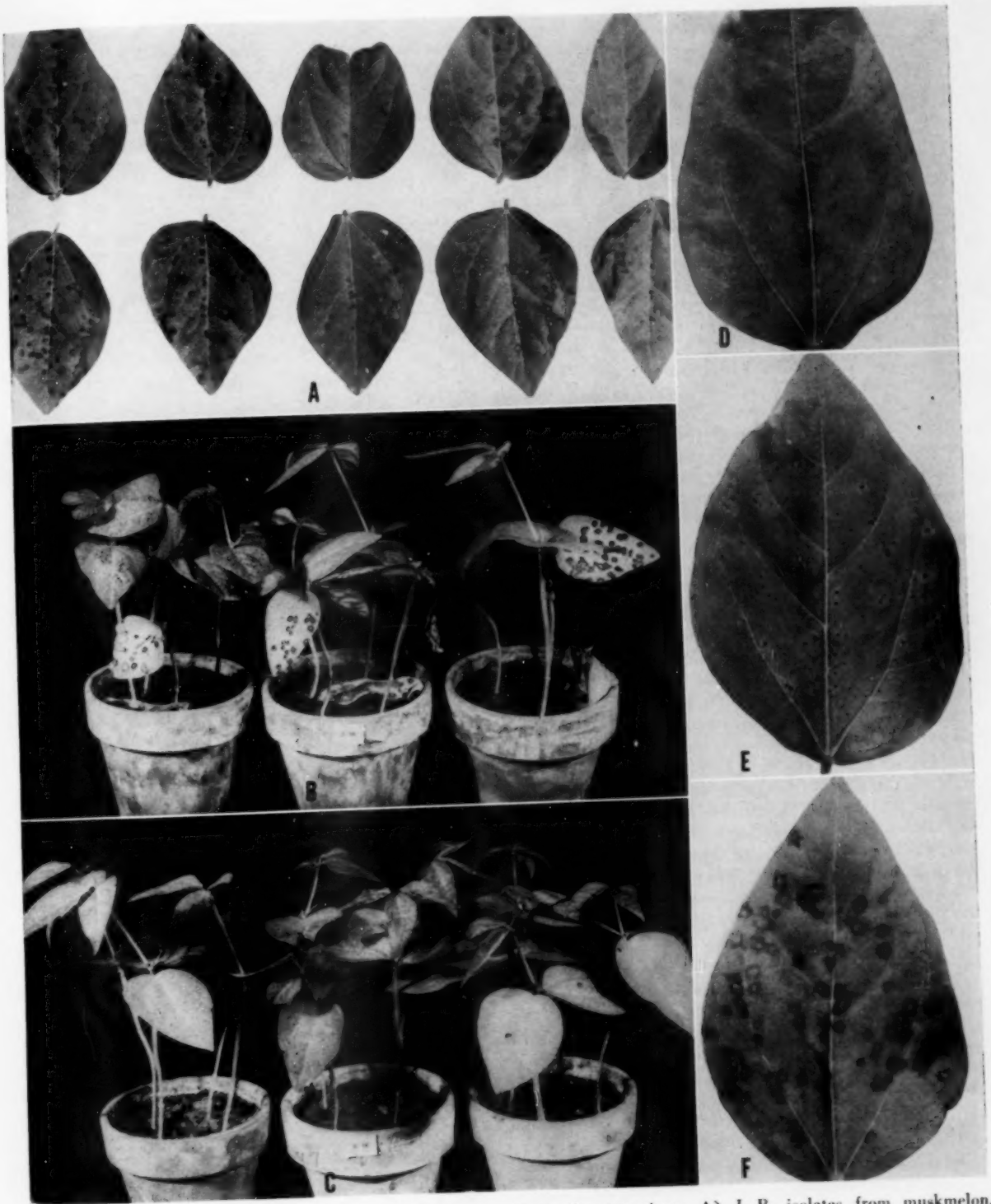

Fig. 1. Local-lesion reactions of Black cowpea to tobacco ringspot virus. **A)** L–R, isolates from muskmelon, cucumber, tobacco, watermelon, and tulip on a susceptible line. **B)** Backcross to susceptible parent. Segregation for susceptible type of local lesioning with top necrosis, and for F_1 types without top necrosis. **C)** Backcross to resistant parent. None susceptible, but resistant parent and F_1 types both present. **DEF)** Segregating types in an F_2 population: homozygous resistant, heterozygous (F_1) resistant, and susceptible.

TABLE 1.—*Observed and theoretical ratios of tobacco ringspot resistance in Black cowpea selfs and backcrosses*

Generation	Resistant		Susceptible	
	Expected	Observed	Expected	Observed
P_1 (Susc.)	0	0	485	485
P_2 (Resis.)	350	350	0	0
F_1	135	135	0	0
F_2	596	600	199	195
BC P_1	84	83	84	86
BC P_2	324[a]	324	0	0

[a] 187 resembling P_2 and 137 the F_1.

produced 86 susceptible and 83 individuals resembling the F_1 in resistance (Fig. 1-B). Backcrosses to the resistant parent gave 187 resistants of the parent type and 137 resistants of the F_1 type. None were susceptible.

The resistant plants had from few to many small local lesions, and lacked systemic infection under ordinary conditions; the susceptibles had typically large lesions and necrosis of the tops.

To estimate the segregation of the resistance factor, inoculations were made on various F_2 plants of relatively uniform size and condition. The plants classified as resistant were arbitrarily separated into heterozygous and homozygous groups by comparing the severity of lesioning with that of known F_1's inoculated at the same time (Fig. 1-DEF). An occasional plant with the F_1 appearance succumbed to systemic invasion and top necrosis, and was grouped with the susceptibles. An observed ratio of 309 resistant to 134 susceptible was obtained from a theoretical 333:111. The ratio of resistant (both classes) to susceptible is about as expected. However, because of the difficulty in making a precise separation into the two resistant classes (F_1 and parental), reliable data could not be obtained on heterozygous versus homozygous plants. It was also noted that the number and size of lesions were variable. Several conditions, still to be precisely defined, appeared to affect local-lesion reaction materially. Preinoculation light conditions and temperature are probably both involved, but factors of host nutrition may also be important.

Part of the original cowpea seed lot from which resistant individuals were selected for study was mass-selfed in the greenhouse without selection for disease reaction. The progeny were used in an attempt to trace the acquisition of resistance. Inoculation with tobacco ringspot produced a ratio of 298 resistant and 179 susceptible phenotypes. It is interesting to note that the F_3 of a single cross would segregate to exactly that ratio.

DISCUSSION.—The genetic ratios obtained represent a near perfect fit for inheritance of a single gene dominant for resistance. The resistance to tobacco ringspot found in our seed stock apparently originated from a single heterozygous individual of unknown origin. The ratio obtained on a group of plants representing a second increase of the original seed was so close to that expected for F_3 that no other explanation seems apparent.

An attempt was made to distinguish between heterozygous and homozygous resistant individuals in the segregating generation. In general, homozygous plants were characterized by very small lesions in small numbers, whereas heterozygous plants had considerably more lesions of a size approaching the susceptible-type lesions. Individual intergradations in appearance made it difficult to categorize precisely for heterozygosity of the resistant factor. Heterozygous individuals generally did not develop top necrosis, but a few succumbed, although more slowly than susceptible plants. It appears that resistance is not in the form of immunity, but is actually a pronounced hypersensitivity modifiable by the environment, since non-segregating inbreds of the resistant type are also variable in the numbers of local lesions produced.

A search of the literature has revealed no report of a ringspot resistance in cowpea such as reported in this study. The hypersensitive reaction is expected to be useful in environmental studies since it appears to be much more responsive than the usual susceptible reaction. The resistant reaction of cowpea to cucumber mosaic giving local lesions is well known, but its analagous susceptible reaction is a systemic mottle (5, 6). The mode of inheritance appears to be identical with that of resistance to cucumber mosaic (6), but preliminary tests indicate that the ringspot resistance is independent of the resistance to cucumber mosaic.

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XYLEM FORMATION AS A FACTOR IN OAK WILT RESISTANCE[1]

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SUMMARY

Examination of microtome sections from branches of artificially and naturally infected white oaks disclosed a type of resistance to oak wilt. Bands of large, open xylem vessels, formed external to bands of plugged springwood vessels during the growing season in which foliar symptoms appeared, and annual rings formed in succeeding years, were not invaded by the pathogen. Pin Oaks treated with Vancide 51 that had symptoms similar to those on wilting white oaks, formed a broad ring of predominantly open vessels almost identical to the bands in diseased white oak. This may be a chemically induced resistance similar to the natural resistance of white oaks.

INTRODUCTION.—Symptoms of oak wilt disease, incited by *Ceratocystis fagacearum* (Bretz) Hunt, have been observed in Ohio to be quite different on members of the red and black oak group than on members of the white oak group. Red, black, scarlet, and pin oaks usually succumb rapidly, diseased trees often dying within 4–10 weeks of inoculation. White oaks, on the other hand, commonly have scattered foliar symptoms and may live for several years. Parmeter, Kuntz, and Riker[3] reported a similar phenomenon in Wisconsin: red and pin oaks wilted rapidly and died within a single growing season, whereas bur and white oaks had foliar symptoms on scattered branches and often survived for more than one year, some actually recovering from the disease. In histological sections new annual rings were observed external to the plugged xylem vessels of diseased bur oaks. When the new annual rings were not invaded, the diseased areas of the xylem were buried and the pathogen died. Of 365 wilting red oaks from which the pathogen was isolated in 1952–1956, according to Jones,[4] only 3 trees were still alive in 1957 and all three had foliar symptoms that year. Of 97 white oaks found to be diseased in 1952–1956, 26% were living but had wilt symptoms in 1957, 19% were dead, and 55% were alive with no wilt symptoms.

Plugging of xylem vessels by gums and tyloses is considered by many workers to be the principal cause of foliar wilt symptoms. If this is true, examination of histological sections should reveal some differences between diseased red and white oaks in the pattern of vascular plugging.

The present work was undertaken to study the morphological basis, if one exists, for the greater resistance of white oaks to oak wilt.

MATERIALS AND METHODS.—Histological sections of oak branches were cut, at 40 μ, with a sliding microtome. Since woody sections tend to curl and split while being cut, a steam-generating apparatus was attached to the microtome so that a jet of live steam could be directed onto the surface of the sample being sectioned, as suggested by Johansen.[5] This served the dual purpose of softening the wood for easy cutting, and preventing the development of air bubbles in the sections. Sections were flooded with 1% aqueous solution of Safranin O for 20 seconds, rinsed with distilled water, flooded with 1% Picro-Aniline blue for 20 seconds, and rinsed with distilled water until the excess stain was removed. Xylem tissues stained red, phloem tissues orange, cork tissues brown, tyloses brownish-yellow, and gums and resins dark red.

OBSERVATIONS AND RESULTS.—Several five-year-old white oaks were inoculated by placing drops of a conidial suspension of the oak wilt pathogen in a cut on the bole of the tree. Inoculations were made on May 8, 1957, and scattered foliar symptoms appeared 1 month later, but the trees remained alive throughout the season, as often happens with diseased white oaks. When branches from these trees were sectioned, a clearly defined band of vessels had formed external to the band of plugged springwood vessels (Fig. 1-A). Examination of tangential sections disclosed open xylem vessels in the external band, formed later in the season, with extensive plugging in the underlying springwood (Fig. 1-CD). The current annual ring of xylem tissue appeared normal in transverse sections of healthy white oaks of the same age (Fig. 1-B). Examination of several branches from the inoculated white oaks on which no symptoms appeared established that both outer bands, constituting an annual ring, were formed during the same growing season. The literature disclosed no previous report of this phenomenon.

Symptoms on the artificially inoculated white oaks were similar to those found on naturally infected trees, i.e. the progress of the disease was considerably slower than in red or pin oak, not all branches had

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[3] Parmeter, J. R. Jr., J. E. Kuntz, and A. J. Riker. 1956. Oak Wilt Development in Bur Oaks. Phytopathology 46: 423-426.

[4] Jones, T. W. 1958. Mortality in Wilt Infected Oaks. Plant Disease Reptr. 42: 552-553.

[5] Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., New York. 523 p.

symptoms, considerable suckering occurred, and the trees were still alive at the end of the season even if inoculated in the early spring. To determine whether the apparently healthy xylem formed external to plugged vessels was an isolated phenomenon or occurred in nature, branches from 3 naturally infected white oaks on which symptoms had appeared within the last three years were sectioned and examined. In each case, a broad band of open vessels was found external to a band of plugged vessels, both bands being formed during the growing season in which symptom appearance had been recorded (Fig. 2). Calculation of the percentage of vessels containing tyloses in the plugged and open bands, based on actual counts, disclosed that although 37–77% of the vessels in the plugged bands contained tyloses, less than 1% contained tyloses in the open bands, indicating restriction of the pathogen to the plugged bands. The pathogen was isolated from all 3 white oaks at the time of symptom appearance, but in at least one case the pathogen could not be reisolated in succeeding years.

In field trials of systemic chemicals, a seven-year-old pin oak was treated with 1500 ml of 1000 ppm Vancide 51, applied as a bole injection, and inoculated 2 days later, on August 9, 1956. The first foliar symptoms appeared in 43 days, but the tree lived throughout 1957. Foliar symptoms in 1957 were scattered, and

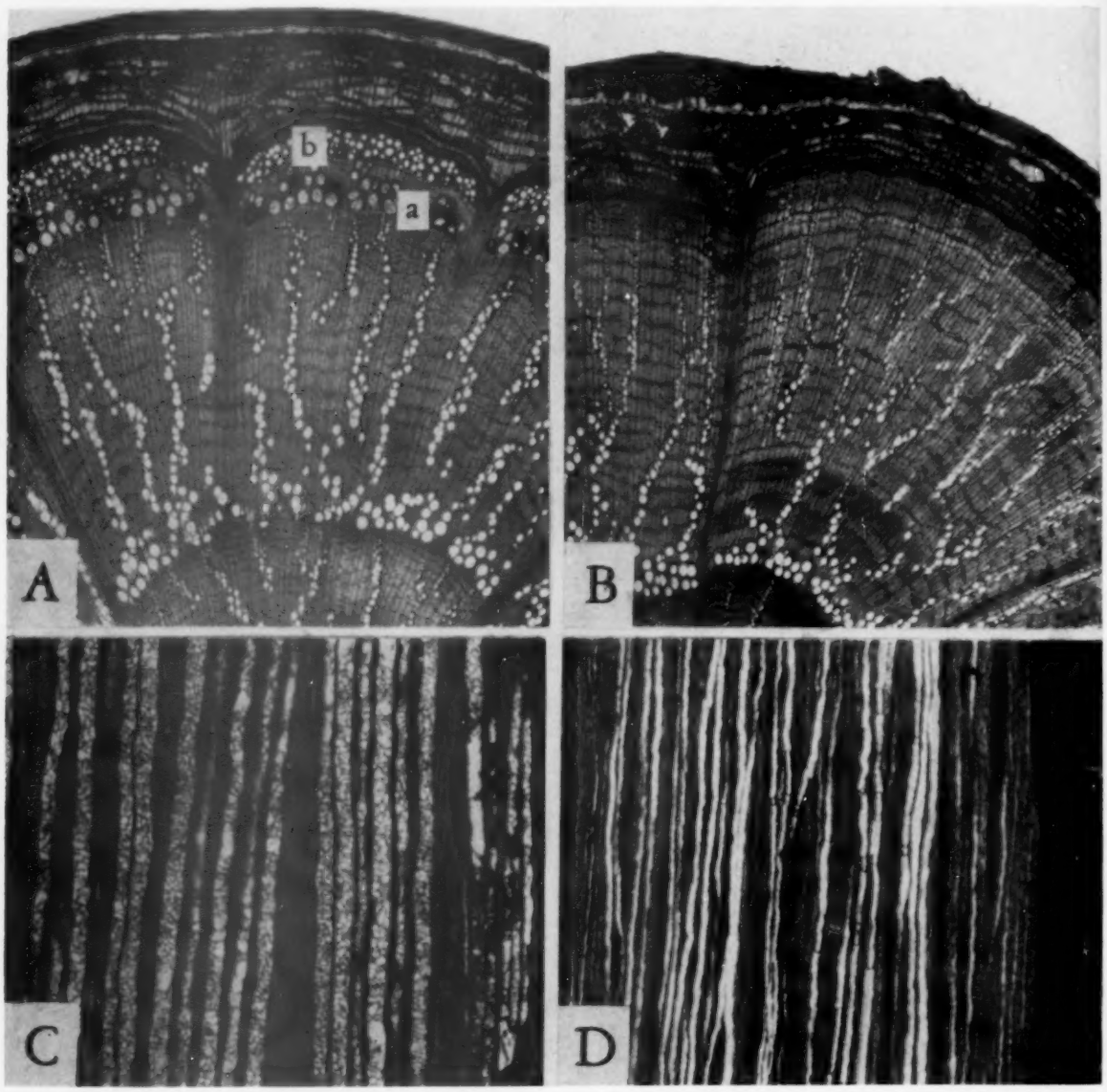

Fig. 1. Resistance in artificially inoculated white oak. **A)** Transverse section through a branch of white oak inoculated on 5/8/57. Section made on 3/8/58, ×10. **B)** Transverse section through a branch of healthy white oak, ×10. **C)** Tangential section through band *a* of inoculated white oak showing plugged vessels, ×11.5. **D)** Tangential section through band *b* of inoculated white oak showing open vessels, ×11.5.

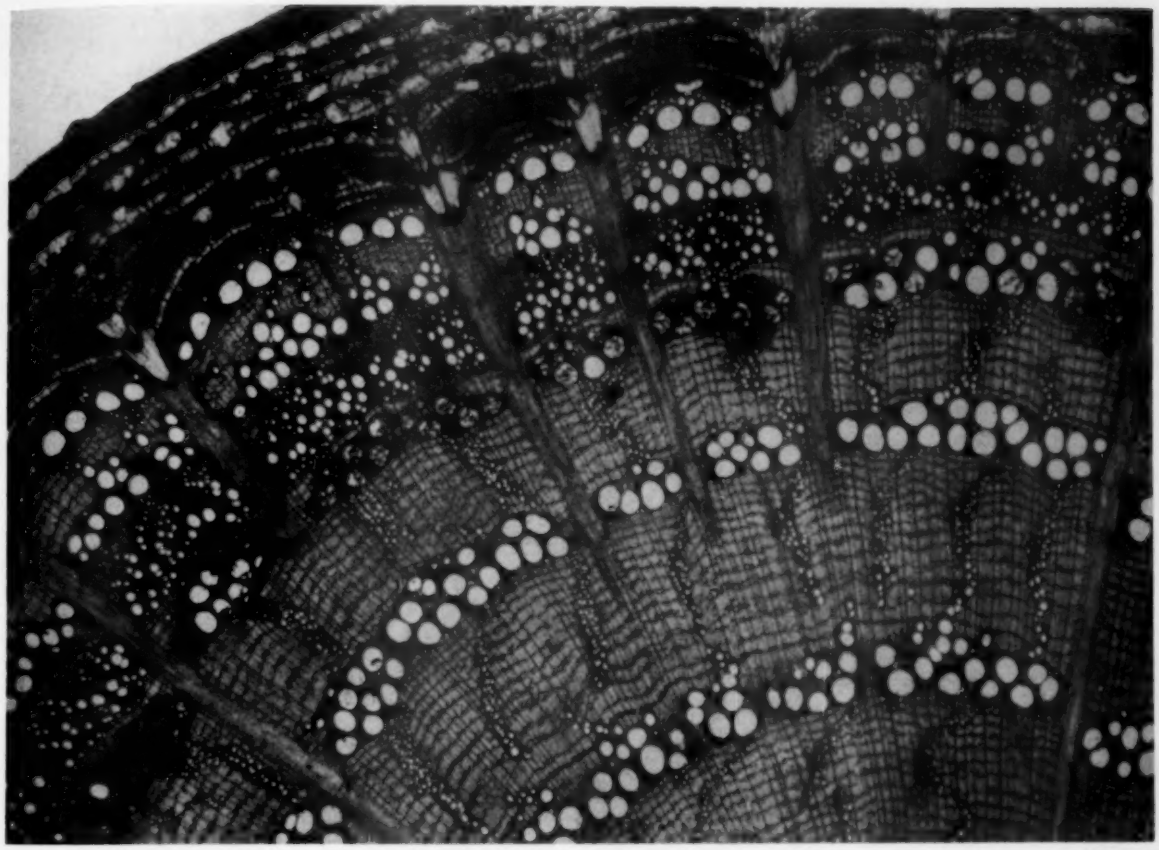

Fig. 2. Resistance in naturally infected white oak. A transverse section through a branch of diseased white oak sectioned on 5/9/58. Double band formation is apparent in the third annual ring. ×11.5.

many suckers appeared, resembling a diseased white oak. Transverse sections from branches of this tree disclosed a ring of open vessels almost identical to the bands of open vessels found in white oaks. Further investigation disclosed that this ring was not formed in 1956, but was the entire xylem growth of the 1957 season. In tangential sections made through the new growth, open vessels were predominant, although many more tyloses were found than in the open bands in white oaks, suggesting probable invasion by the pathogen or a diffusion of its metabolic products into at least some of the new growth. This type of growth ring was not observed in transverse sections of healthy pin oak. Examination of sections from a pin oak treated with Vancide 51 and inoculated in 1957 disclosed a growth pattern the same as that observed in the 1956 tree. Both trees were still alive in July, 1958, with scattered symptoms present.

Discussion.—The formation of bands of open xylem vessels external to bands of plugged springwood vessels during the same growing season in white oaks infected by the oak wilt fungus is a phenomenon not previously reported. The absence of gums and tyloses in these open bands is evidence that the pathogen is restricted to the plugged bands of springwood; in at least one case the pathogen could not be reisolated in succeeding years following symptom appearance. A sharp line of very small cells is formed between the infected vessels and the apparently healthy tissues formed later in the season. This line of cells may serve as a barrier in isolating the pathogen from the newly formed tissues and may be considered, in white oak, a type of resistance to oak wilt. The cause of this double band formation is not known, but it is undoubtedly promoted by the pathogen or a metabolic product of the pathogen, since no double bands were found in trees containing wound-induced tyloses only.

Since pin oaks treated with Vancide 51 are still alive several years after inoculation, and a wide band of predominantly open vessels was found as in white oak, the formation of this band may be thought of as a type of resistance in pin oaks induced by the application of Vancide 51. Further experimentation with Vancide and related compounds may lead to the development of a practical control measure for oak wilt.

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A NEW STRAIN OF RING POX VIRUS IN ROYAL APRICOT[1]

George Nyland and H. E. Thomas[2]

SUMMARY

A graft-transmissible disease is reported on Royal apricot, with leaf, fruit, and (sometimes) petiole symptoms resembling apricot ring pox. Fruit symptoms consisted of dark rings, arcs, and spots of necrotic tissue much more superficial than ring pox on other varieties. The disease is more severe in Royal than in Tilton. The resemblance to apricot ring pox suggests that this disease may be caused by a new strain of ring pox virus that is capable of inducing symptoms on Royal.

A disease of Royal (Blenheim) apricot in the Niles area (Alameda County, California) was called to our attention in May, 1954. It resembled apricot ring pox as described in the literature on Tilton and some other apricot varieties (1, 2). Symptoms of ring pox have not previously been described on Royal, although this variety has been reported as a symptomless carrier (3). Since the disease on Royal in the Niles area showed similarities to some strains of peach mosaic on Royal in southern California, transmission tests to peach were immediately tried, but no symptoms were produced. Considering the similarity of the symptoms of the Royal disease to those of apricot ring pox, this virus may be a strain of apricot ring pox virus that is capable of inducing symptoms on Royal.

SYMPTOMS.—Leaf symptoms of the disease on Royal apricot consist of chlorotic spots, usually becoming necrotic after a time, and line patterns and chlorotic rings in various forms, but more or less irregular and angular in shape. The most conspicuous symptoms in inoculated Tilton were chlorotic areas and subsequent shotholing, and in Royal, despite considerable tattering and shotholing, chlorotic lines and rings and line patterns (Fig. 1). Symptoms were most severe on leaves produced in early spring. Leaves produced in late spring and summer were symptomless at Davis, California. In Alameda County and Berkeley, however, symptoms were produced on summer leaves as well as on leaves produced earlier. In the 1958 season at Davis, when leaf symptoms were especially severe, inconspicuous petiole symptoms also occurred: small, scabby lesions that caused petioles to twist and curve slightly. This symptom was not in all diseased trees, and was more apparent in Royal than in Tilton.

Fruit symptoms varied in severity in different years. They were more conspicuous at Davis than in the Niles area. At Davis, symptoms on the fruits began to show about the middle of April. The first evidence of fruit lesions was small, water-soaked spots, arcs, or rings in the epidermis, enlarging with the fruits and becoming reddish-brown or purple (Fig. 1). As the season advanced the areas became necrotic and scabby. In some the scabby tissue shrank away or dropped out, leaving superficial pits. Most spots and rings were superficial, blemishing only the surface. As the fruit colored, many of the blemishes became difficult to detect. Others remained fairly conspicuous, giving the fruit a "dirty" appearance. The fruit symptoms were much milder and more superficial than the symptoms of typical apricot ring pox. Symptoms were generally milder on Tilton fruits than on Royal.

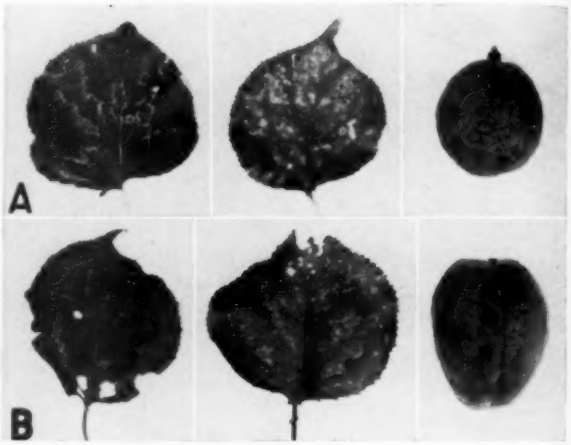

Fig. 1. Leaf and fruit symptoms of a Royal strain of apricot ring pox virus. **A)** Royal; **B)** Tilton.

TRANSMISSION TESTS.—Transmission was first attempted on May 7, 1954, from affected orchard trees of Royal apricot. At Davis, buds and bark chips were set in apricot trees of Royal and Tilton and in peach trees of the varieties Elberta, Rio Oso, and J. H. Hale; at Berkeley, scions were grafted to peach seedlings growing in pots in a greenhouse. Leaves on shoots that had developed from inoculation buds and scions showed typical symptoms in 1955 at Davis and Berkeley. In April, 1955, one Royal tree at Davis showed symptoms on a few leaves on a fruit spur near an inoculation point. No further evidence of transmission was obtained that year. In 1956, characteristic and abundant symptoms were produced in all apricot trees inoculated in 1954. Symptoms were evident 6–8 feet above inoculation sites in the main branches of Royal trees, but only 2–3 feet from inoculation sites in Tilton

[1] Accepted for publication December 19, 1958.

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trees. In Royal (Blenheim) trees, symptoms were evident within 6 in. of the actively growing shoot terminals in May. By 1957, symptoms showed throughout the Royal trees in leaves and fruits, but symptoms in Tilton were still restricted within 6 feet of inoculation sites. No symptoms were seen on any of the inoculated peach trees in 4 years.

Additional transmission tests to apricot were made in 1955 and 1956. In each case, evidence of transmission was obtained in the second growing season after inoculation, which is typical for ring pox.

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CHEMICAL AND BIOLOGICAL STUDIES ON A REACTION BETWEEN CAPTAN AND THE DIALKYLDITHIOCARBAMATES[1]

Raymond J. Lukens[2]

SUMMARY

Captan—*N*(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide—will react with sodium dimethyldithiocarbamate (NaDDC). Captan is destroyed. NaDDC is converted to tetramethylthiuram disulfide (TMTD) and tetramethylthiuram monosulfide (TMTM). Chloride ion and carbon disulfide are produced, but no hydrogen sulfide has been detected. The reaction mixture is as toxic as NaDDC to the growth of *Saccharomyces pastorianus*. The toxicities of NaDDC, TMTD, and TMTM were consistent with expected concentrations of these materials in the reaction mixture. $Zn(DDC)_2$ and $Fe(DDC)_3$ react with captan in the same manner, but much more slowly. The reaction of DDC^- with captan appears to be similar to that of other sulfhydryl compounds. Bis(dimethylthiocarbamyl) trithiocarbonate, an addition product of DDC^- and the thiocarbonyl fragment derived from captan, probably is formed and then is decomposed to TMTM and carbon disulfide. However, the intermediate compound is too unstable to be detected. Thus the DDC portion contributes a sulfur atom to the carbon disulfide resulting from this decomposition. Yeast cells may act like DDC^- in that they may contribute sulfur to the carbon disulfide produced when they are treated with captan.

Sulfhydryl compounds react with captan—*N*(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide—to destroy its fungitoxicity (5). In this reaction the dialkyl dithiocarbamates may produce sulfhydryl-containing compounds or their corresponding ions. Because captan might be mixed with fungicides of this type, it is important to know if these compounds react. If so, are the products of the reaction as fungitoxic as the reacting materials?

MATERIALS AND METHODS.—*Chemical studies.*—Sodium dimethyldithiocarbamate (NaDDC) was chosen as the form of the alkyldithiocarbamate because it readily ionizes to form DDC ions. Recrystallized captan was generously supplied by the California Spray Chemical Company, Moorestown, New Jersey. Ziram ($Zn(DDC)_2$) and ferbam ($Fe(DDC)_3$) were made by precipitation of the metal salts upon addition of the metal sulfate to an aqueous solution of NaDDC. Stauffer Captan 50W (source 1956), Zerlate (76% ziram), and Fermate (76% ferbam) were used in experiments employing commercial formulations.

Chloride ion was determined by the Volhard method (7). Captan and NaDDC were dissolved in ethylacetate and stirred for 1 hour. Essentially all of the chloride ion was recovered in the precipitate. The precipitate was dissolved in 40 ml of water, and 3 aliquots were analyzed for chloride ion. Aqueous suspensions of captan and $Zn(DDC)_2$ or $Fe(DDC)_3$ were aged 4 days on a shaker at 30°C. Triton B-1956, 0.02% was added to the mixture to improve the wetting properties. The supernatant was adjusted to 100 ml, and 3 aliquots of 25 ml each were analyzed for chloride ion. Aqueous suspensions of Captan 50-W and Zerlate or Fermate were shaken 8 days at 30°C. The supernatant was analyzed for chloride ion as in the earlier experiment.

Carbon disulfide and hydrogen sulfide were respectively collected in Viles' reagent (8) and zinc acetate solution from the reaction of captan and NaDDC in 95% ethanol. The resulting gases were collected for 4 hours. Copper dimethyldithiocarbamate in Viles' reagent was determined in a Coleman colorimeter at 430 mμ, and hydrogen sulfide was determined iodometrically in zinc acetate.

One millimole of captan was suspended in 100 ml of 30% ethanol buffered (pH 6) with 0.5 millimole potassium phosphate, and 4 millimoles NaDDC were

[1] Accepted for publication December 22, 1958.
[2] Assistant Plant Pathologist.

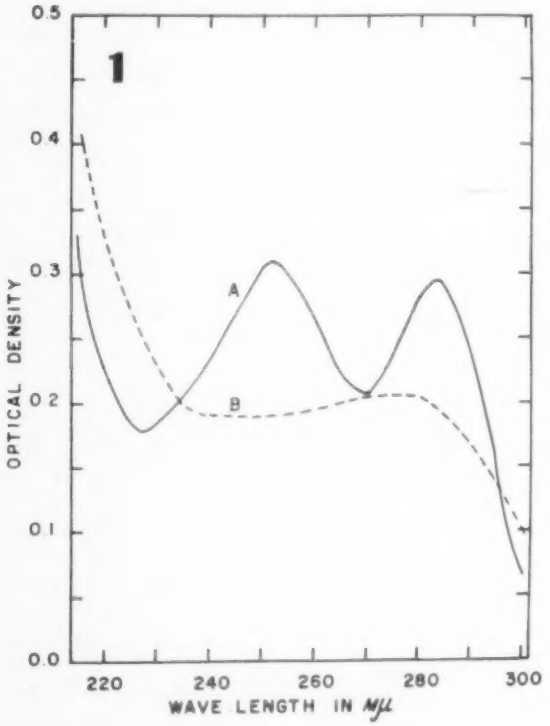
added to the stirred suspension. After 1-hour reaction time, the organic products were separated. A white precipitate (precipitate 1) was removed by centrifugation and washed with 95% ethanol. The yellow supernatant was distilled in vacuo to remove the ethanol, whereupon a yellow precipitate appeared. This was redissolved in ethanol and filtered, and, upon evaporation of the solvent, yellow needle crystals were recovered (precipitate 2). The pale-yellow aqueous phase remaining was extracted 4 times with ethylacetate. The water fraction containing sodium chloride and phosphate salts was discarded. The yellow ethyl acetate fraction was extracted 4 times with 10% sodium carbonate and dried down, and yellow needles were recovered. This fraction is the same as precipitate 2, which probably contained some unreacted NaDDC. The sodium carbonate fraction was acidified, and extracted 4 times with ethylacetate. The water fraction was discarded. The ethylacetate fraction was dried down and designated precipitate 3.

Ultraviolet absorption spectra were determined with the Beckman DU spectrophotometer. Ascending paper chromatography technique was used with Whatman #1 paper. The developing solution was ethanol-water-ammonia (10:10:1 by volume). Melting points were determined with a Fisher-Johns melting-point apparatus.

Biological studies.—The toxicity of the various compounds and the mixture of NaDDC and captan were determined on *Saccharomyces pastorianus* Hans. Appropriate stock solutions of the compounds were made in 95% ethanol. Two-tenths ml of the stock solution was diluted in 20 ml total volume of experimental culture. The cultural medium was the same as that of Lukens and Sisler (5). The initial inoculum of 0.058 mg (dry wt.) per ml was taken from rapidly growing cells of a 24-hour-old culture. The test cultures were incubated on a shaker in a water bath at 30°C. Optical density of 24-hour-old cultures was read on a Coleman colorimeter using the 470 mμ wave-length filter.

RESULTS.—*Chemical studies.* NaDDC has a characteristic ultraviolet absorption spectrum that disappears when it is mixed with captan (Fig. 1). There is evidently a reaction between these two fungicides. Chloride analyses (Table 1) indicate that 4 moles NaDDC almost completely decompose 1 mole captan in 1 hour. Thus, like other sulfhydryl compounds, NaDDC reacts with captan.

During the reaction, the odor of carbon disulfide was detected. From a reaction of 1×10^{-5} mole captan and 4×10^{-5} mole NaDDC, there evolved 0.46×10^{-5} mole carbon disulfide in 4 hours. The sources of carbon disulfide in this reaction will be considered



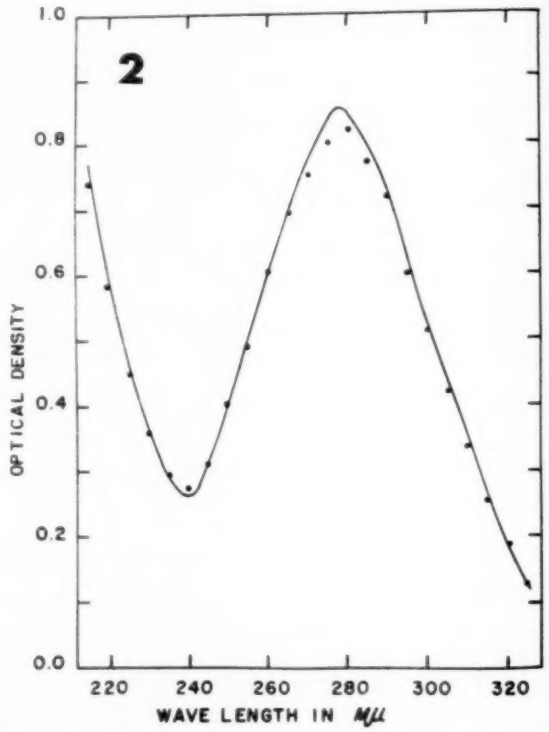


Fig. 1–2. Fig. 1. Ultraviolet absorption spectrum of the reaction mixture of 0.1 millimole of captan and 0.4 millimole of NaDDC in 95% ethanol. The solutions were diluted 1:10 with 95% ethanol before ultraviolet-absorption measurements were made. Curve **A)** 0.4 millimole of NaDDC: **B)** 0.1 millimole of captan plus 0.4 millimole of NaDDC after 1 hour. **Fig. 2.** Ultraviolet absorption spectrum of precipitate 2, obtained from the reaction of captan and NaDDC in 30% ethanol, compared with TMTM. The points represent the spectrum of 10 ppm precipitate 2 in 95% ethanol.

TABLE 1.—*Release of chloride ion in the reaction of captan and NaDDC in ethylacetate*

Captan[a] to NaDDC (molar ratio)	Moles of chloride ion per mole of captan in 1 hour
1:0	0.04
1:1	0.75
1:2	1.53
1:3	2.14
1:4	2.70
1:5	3.06
0:5	0.18

[a] One-tenth millimole of captan was used in each sample. NaDDC = sodium dimethyldithiocarbamate.

later. No hydrogen sulfide could be detected.

The organic compounds isolated from the reaction mixture were identified in the following manner. The melting points of precipitate 1, of tetramethylthiuram disulfide (TMTD) and of a mixture of the two were all in the range 143–145°C (Table 2). Moreover, the ultraviolet absorption spectrum of the precipitate was the same as that of TMTD in a 95% ethanolic solution of 10 ppm of the samples. Precipitate 1 is therefore considered to be TMTD.

Precipitate 2 was identified as tetramethylthiuram monosulfide (TMTM) (Table 2; Fig. 2) in the same manner. Moreover, the infrared absorption spectrum was the same for precipitate 2 and TMTM. The Rf values of precipitate 2 and TMTM were compared by ascending paper chromatography in a developing solution of ethanol-water-ammonia (10:10:1 by volume). Both had a value of 0.8. When a mixture of the two compounds was chromatographed, only 1 spot (Rf 0.8) was detected under ultraviolet light. These spots were eluted and the ultraviolet absorption of their solutions were similar to those reported in Fig. 2. The melting point of the precipitate (100–104°C.) was lower than that of TMTM (106–107°C.), which suggests that precipitate 2 is TMTM, because the mixed melting point (104–105°C.) was higher than that of the precipitate. The mixture was evidently purer than the precipitate alone. TMTD and TMTM are apparently products resulting from the reaction of NaDDC and captan.

The melting point of precipitate 3 and its mixed melting point with tetrahydrophthalimide are 133–134°C. (Table 2). Because the mixed melting point

TABLE 2.—*Melting-point determinations of materials obtained from the reaction of captan and NaDDC with known compounds*

Sample	Melting point (°C)
Precipitate 1	143–145
TMTD	145
Mixture	143–145
Precipitate 2	100–104
TMTM	106–107
Mixture	104–105
Precipitate 3	134
Tetrahydrophthalimide	134
Mixture	133–134

TABLE 3.—*Toxicity of captan plus NaDDC (1:4 molar ratio) to Saccharomyces pastorianus in liquid shake culture*

Treatment[a]	% growth inhibition at various doses at 24 hours				
	x	3.3x	6.7x	10x	100x
Captan[b]	11	21	27	25	96
NaDDC[c]	33	91	98	100	95
1:4 Molar ratio[d]	32	93	99	100	99

[a] The final concentration of the solvent in the culture was 1%.

[b] x dose of captan was 3×10^{-5} molar.

[c] x dose of NaDCC (sodium dimethyldithiocarbamate) was 12×10^{-5} molar.

[d] x dose of 1:4 molar ration was captan 3×10^{-5} molar and NaDDC 12×10^{-5} molar. Captan and NaDDC were mixed in 95% ethanol and after 1 hour were added to the culture as noted in footnote a.

was the same as that of the two samples, precipitate 3 is considered to be tetrahydrophthalimide.

When NaDDC is held under conditions similar to those of the experiment, no TMTD or TMTM can be detected.

The extent to which the reaction of captan and $Zn(DDC)_2$ or $Fe(DDC)_3$ occurs in water was determined. First, 0.5 millimole of captan was suspended with 2.0 milliequivalents of $Zn(DDC)_2$ or $Fe(DDC)_3$ in 50 ml water. In 4 days, 11.0% of captan decomposed with $Zn(DDC)_2$ added, 5.1% with $Fe(DDC)_3$ added, and 2.8% without additions. In a second experiment, 1 g Captan 50W was suspended with 1 g Zerlate or 1 g Fermate in 50 ml water. In 8 days, 8.0% of captan decomposed with Zerlate added, 4.0% with Fermate added, and 2.0% without additions. At the termination of the experiments, the pH was about 5.0 in all mixtures.

Biological studies.—It was of interest to determine the effect of the reaction on the fungitoxicity of the two fungicides. The fungicides were mixed in ethanolic stock solutions 1 hour before being added to the cultural medium. The reaction products are apparently as toxic to *S. pastorianus* as is NaDDC (Table 3). The toxic effects due to captan are obscure in the reaction.

The toxicities to *S. pastorianus* of captan, NaDDC, TMTD, TMTM, and precipitates 1 and 2 were compared. From LD 50 values on a molar basis from the data in Table 4 (captan, 6×10^{-7} molar; NaDDC 6×10^{-7}; TMTD, 4×10^{-8} molar; and TMTM, 14×10^{-7} molar), captan and NaDDC are found to be equally toxic, with TMTD more toxic and TMTM less toxic. However, all of the dithiocarbamates have a steeper dosage-response curve than captan. NaDDC and TMTD exhibited, slightly, the zone of inversion reported for NaDDC by Goksyr (2) and for TMTD by Dimond et al (1). When media containing precipitate 1 or TMTD were inoculated with yeast and examined after 48 hours, no growth was observed in cultures containing 0.3 or 3 ppm of either sample, but a concentration of 1 ppm permitted growth about equal to that of the control. The inversion zone would possibly

TABLE 4.—*Toxicity to Saccharomyces pastorianus in liquid shake culture of captan, NaDDC, precipitate 1, TMTD, precipitate 2, and TMTM at various concentrations*

Concentrations[a] (ppm)	% growth inhibition at 24 hours					
	Captan	NaDDC	Ppt.[b] 1	TMTD	Ppt. 2	TMTM
0.01	11	0	55	55		
0.02			87	88		
0.03	21	11	97	98	6	3
0.1	41	98	100	100	53	24
0.3	71	100	100	100	98	61
1.0	99	93	98	99	100	100
3.0		100	100	100	100	100
Molecular weight	300	143		240		208

[a] The fungicides were added to 95 percent ethanol. The final concentration of the solvent in the culture was 1 percent.
[b] Precipitate.

be more evident if small increments in concentration of NaDDC or TMTD were taken on either side of 1 ppm.

The toxicities of precipitate 1 and TMTD are similar, if not identical, as they would be if TMTD is formed in the reaction. In the case of precipitate 2 and TMTM, the toxicities are quite similar, but precipitate 2 is more toxic at partially inhibitory concentrations. This may be caused by a trace of NaDDC or TMTD in the precipitate. On the whole, the biological data are in agreement with the chemical data suggesting that TMTM is formed in the reaction.

DISCUSSION.—The proposed pathway for the reaction between captan and NaDDC is as follows:

(1) captan + NaDDC → Na-tetrahydrophthalimide + NaCl + TMTD + thiophosgene

(2) thiophosgene + 2 NaDDC →

$$[(CH_3)_2\,N{-}\overset{\overset{S}{\|}}{C}{-}S{-}\overset{\overset{S}{\|}}{C}{-}S{-}\overset{\overset{S}{\|}}{C}{-}N(CH_3)_2] + 2\ NaCl$$

(3) $[(CH_3)_2\,N{-}\overset{\overset{S}{\|}}{C}{-}S{-}\overset{\overset{S}{\|}}{C}{-}S{-}\overset{\overset{S}{\|}}{C}{-}N(CH_3)_2] \rightarrow$ TMTM + CS_2

This is similar to that proposed for the reaction between captan and cysteine (5). In contrast to cysteine, thiophosgene has only 1 available reaction site on DDC^-. In this case, the addition product is, therefore, an open chain instead of a ring compound. The addition product, bis(dimethylthiocarbamyl)-trithiocarbonate is probably unstable, because it was not detected and TMTM was recovered from the reaction mixture. TMTM is probably formed from the decomposition of this addition product.

Carbon disulfide may arise from three possible sources: 1) decomposition of DDC^- ions, 2) thiophosgene, and 3) decomposition of the addition product from thiophosgene and NaDDC. All of the sulfur in carbon disulfide was previously thought to arise from the sulfur of captan when it reacts with sulfhydryl compounds (5). The recovery of TMTM from the reaction mixture of captan and NaDDC indicates that part of the sulfur comes from the sulfhydryl compound after an unstable addition product is formed by the combination of thiophosgene with a sulfhydryl compound. A portion of the carbon disulfide formed in yeast cells treated with captan (5), might arise from an unstable addition product of a sulfhydryl

$$\overset{\overset{S}{\|}}{C}$$

group of the cell and the thiocarbonyl group (—C—) derived from the decomposition of captan.

Why is hydrogen sulfide not detected as a reaction product? Thiophosgene should give rise to H_2S from a secondary reaction with water. However, the H_2S so liberated might never appear, because it can react readily with other components of the system: captan, NaDDC, and possibly TMTD. Hydrogen sulfide has been found to react with captan in a manner similar to other sulfhydryl compounds (unpublished data).

When NaDDC is converted to TMTD and TMTM by captan, toxicity of these compounds toward yeast equals or exceeds the toxicity of pure NaDDC. The inversion phenomenon displayed by NaDDC, 12×10^{-6} molar (Table 3), is less pronounced in the reaction mixture. A reduction in concentration of DDC^- ions by the reaction would account for this effect.

TMTM is quite toxic to growing yeast cells. Klöpping (3) and Klöpping and van der Kerk (4) found it to be less potent than NaDDC and TMTD, but ascribed its toxicity to the formation of DDC^- ions. Goksøyr (2) found that TMTM hydrolyzes very slowly to DDC^- ions, but is removed quite rapidly from the ambient fluid by yeast cells. Tests of the ability of TMTM to inhibit enzymes *in vitro* and respiration of resting yeast cells have shown that the fungicide is less active than TMTD and DDC metal complexes (2). Miller and Elson (6) found that TMTM is quite toxic to growth of fungi and bacteria. Perhaps TMTM would be a more desirable alkyldithiocarbamate than TMTD and NaDDC to use in determining the vital sites in cells that are affected in the inhibition of cell growth by that group of fungicides. However, TMTM may be toxic in its own right in addition to hydrolyzing to DDC^- ions. This phenomenon could account for its effectiveness at low concentrations.

Presumably, when captan is mixed with a metal dimethyldithiocarbamate in aqueous suspensions, the reaction proceeds slowly because of 1) the low solubilities of the components and 2) the ionization of the dithiocarbamates. If such a combination spray is employed in the field, a residual mixture of captan, DDC-metal, TMTD, and TMTM would eventually be formed. Such an array of fungicidal potency may be

desirable for controlling several fungal disorders.

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SOME HOST AND VECTOR RELATIONSHIPS OF STRAINS OF THE BARLEY YELLOW-DWARF VIRUS[1]

H. V. Toko and G. W. Bruehl

SUMMARY

Two vector-specific isolates of the barley yellow-dwarf virus were detected among 34 field collections tested. Using the apple grain aphid *(Rhopalosiphum fitchii)* and the English grain aphid *(Macrosiphum granarium)*, 32 isolates were transmitted by both aphids, 1 isolate (from volunteer barley) by the apple grain aphid only (AG strain), and 1 isolate (from wild oats) by the English grain aphid only (EG strain). Both vector-specific strains produce symptoms typical of barley yellow-dwarf on oats, wheat, and barley, including mottling, discoloration, and leaf serrations. Dwarfing and suppression of tillering are more severe in AG-infected oats and barley than EG-infected plants. Cross-protection tests failed to show interference between the 2 vector-specific virus entities, even when symptoms of the first were visible before the second was introduced. When both strains were inoculated simultaneously into young oats, no alteration in symptoms over those produced by the more virulent AG strain alone was observed. The feeding periods of the respective vectors for the acquisition and inoculation of the vector-specific strains are quite similar. Acquisition feeding of 24 hours followed by inoculation feeding of 4–8 hours is necessary for infection to be more than rare. Lengthening either feeding period increases the efficiency of transmission by both vectors. All nymphal instars and adults[2] of both aphids are effective vectors.

The barley yellow-dwarf virus has several aphid vectors (7, 14, 15). Workers are in agreement that the yellow-dwarf virus is persistent in its vectors (1, 7, 11, 15), that some strains of the virus are better adapted to transmission by one grass aphid than another (9, 10, 12, 13), that many strains are adapted to transmission by several aphid species (3, 7, 13, 15), and that there is no interference among strains of the yellow-dwarf virus within the host (1, 13) or within the aphid (1). The present study was initiated to elucidate some of the relationships to the barley yellow-dwarf virus of 2 of the vectors, the English grain aphid (*Macrosiphum granarium* Kirby) and the apple grain aphid (*Rhopalosiphum fitchii* Sand.).

VECTOR SPECIFICITY. — The observation that transmission of a single collection of the barley yellow-dwarf virus was differential by English grain and apple grain aphids (12) led to the speculation that strains of the virus might exist that are adapted to transmission by one of these aphids but not by the other, i.e., vector-specific strains. A search for such strains was made in 1955 and 1956, and infected plants were obtained in the Washington counties Adams (1),[3] Clark (3), Klickitat (1), Pierce (5), Skagit (1), Spokane (10), Walla Walla (1), and Whitman (12).

The plants were dug and transplanted into 6-in. pots and assembled in the greenhouse at Pullman, Washington. Precautions were taken against spread-

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[2] In the case of the apple grain aphid, the term "adult" herein applies either to adults reared on the secondary host (oats in this case) or to the summer viviparae.

ing or mixing the virus in transit. The collection was periodically fumigated thereafter.

Virus was recovered from these field collections by means of virus-free aphids obtained by the method of Oswald and Houston (6). The virus-free aphids were increased and maintained on healthy Fulghum oats (*Avena byzantina* Koch, C.I. 708). Several adult[2] virus-free aphids of each species, placed simultaneously on a source plant, were allowed 36 hours of acquisition feeding and then visually separated. The adults of each species were placed individually, 1 per plant, on 10 plants for each aphid species. Fulghum seedlings about 2–4 in. tall were used as assay plants. The aphids were killed after a 48-hr. inoculation feeding period. The seedlings were grown and observed for subsequent disease development. Each collection was tested at least twice in this manner. Aphids from the stock colony were allowed to feed on check plants in each test to make certain they were non-viruliferous.

The viruses of most of the plant collection were transmitted by both species. However, 2 of the 34 original plants were found infected by a virus transmitted by only 1 of the aphid test species. A volunteer barley (*Hordeum vulgare* L.) from near Goldendale was infected by a strain transmitted only by the apple grain aphid (AG strain). A second collection of volunteer oats south of Colfax carried a strain transmitted only by the English grain aphid (EG strain).

The vector-specificity of these 2 collections was checked through 6 successive attempted transmissions, using 4 adults on each of 20 plants for each aphid species in each trial. In no case did the non-vector aphid transmit the strain of the other aphid.

A single isolate from near Dusty, Washington, was initially transmitted by both aphid species. In later tests with this isolate, going back to the original collection plant each time, transmissions by the English grain aphid became progressively fewer until about 4 months after the collection date, when the isolate became AG-specific. All other collections tested for prolonged periods in the greenhouse remained readily transmissible by both species.

The question of whether these vector-specific entities were typical of the yellow-dwarf virus presented itself. Seed transmission and mechanical transmission failed. Inoculations were made on several varieties of cultivated oats (*Avena sativa* L., *A. byzantina* Koch), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and many grasses (4). Typical yellow dwarf developed on all susceptible hosts, reproducing in detail all the symptoms given by Oswald and Houston (6, 7), and no atypical symptoms were seen. The AG strain was more virulent than the EG strain in most hosts, both in greenhouse and field trials.

Vigorous disease development and good symptom expression depended on high light intensity and moderate temperatures, as has been noted for barley yellow dwarf (1, 8, 11, 16). Prolonged periods of dense cloud cover, common in winter in the Pacific Northwest, were attended by faint discolorations, lack of stunting, and even evanescence of symptoms. Periods of excessively high greenhouse temperatures that accompany brilliant sun were likewise detrimental to symptom expression. The full destructive capabilities of the two strains were best seen in the field under normal light and temperatures.

The lack of seed transmission, failure of attempted mechanical transmission, production of all known symptoms on a wide range of hosts, and similar environmental responses all indicated that the AG and EG isolates could best be considered strains of the barley yellow-dwarf virus.

INTERFERENCE STUDIES.—Cross-protection or interference between strains of a virus within a host plant has been used by many workers as a criterion of close relationship between strains. The vector-specificity of the AG and EG strains presented an opportunity to test for cross-protection. The first true leaf of Fulghum oat seedlings in the 2-leaf stage of development was inoculated with the less virulent EG strain of the barley yellow-dwarf virus by means of the English grain aphid. The same leaf of the same plants was then inoculated with the more virulent AG strain of the virus after different intervals: 0, 1, 2, 3, 4, 7, and 14 days. An identical series of tests was set up with viruses inoculated in reverse order. In both series, 4 plants were used for each inoculation time, and each series was repeated 4 times.

As a check series, aphids of both species were fed on the source plants infected with single unmixed vector-specific strains, and transferred to seedlings. These plants were used to determine whether or not the vector-specificity still existed. These singly-infected plants were also used in making symptom comparisons with those of the interference series.

Recovery of virus from the plants inoculated with both vector-specific strains of the yellow-dwarf virus was attempted 3 weeks after the plants had been inoculated with the challenger virus strain. In all recovery attempts, the aphids were allowed 48-hr. acquisition and inoculation feeding periods. In both inoculation and recovery feedings, the aphids were confined to specific leaves by the use of small cages similar to those described by Eide and McLean (5).

No apparent interference occurred between the 2 vector-specific virus strains (Table 1). The number of plants from which both strains could be recovered did not differ, whether the less virulent EG strain was inoculated before or after the AG strain.

Continued transmission of the two virus strains in the manner previously described showed that the vector-strain relationship of the two virus entities still persisted, either in mixed or singly inoculated plants. The strains remained vector-specific.

Sixty days after inoculation with the challenger virus strain, the symptoms typical of the AG strain dominated in all plants that had been inoculated with this strain first. In addition, the symptoms of this

[2] Numbers indicate the number of collections studied from each county.

TABLE 1.—*Recovery of the AG and EG strains of barley yellow-dwarf virus from various leaves of plants following inoculations separated by different periods*

Inoculated virus		Days between inoculations	Virus recovery 21 days after last inoculation					
			Inoculated leaf[a]		Leaf 3		Leaf 5[b]	
Initial	Challenger		AG	EG	AG	EG	AG	EG
Both		0	15/16[c]	12/16			14/16	12/16
AG	EG	1	16/16	13/16			16/16	11/16
EG	AG	1	15/16	12/16			15/16	12/16
AG	EG	2	10/16	12/16			10/16	11/16
EG	AG	2	12/16	12/16			12/16	12/16
AG	EG	3	15/16	14/16			16/16	14/16
EG	AG	3	16/16	15/16			15/16	14/16
AG	EG	4	12/16	14/16			14/16	13/16
EG	AG	4	13/16	11/16			16/16	14/16
AG	EG	7	16/16	12/16	9/10	7/10	15/16	12/16
EG	AG	7	15/16	13/16	10/10	8/10	15/16	13/16
AG	EG	14	15/16	12/16	5/6	5/6	15/16	11/16
EG	AG	14	16/16	14/16	6/6	5/6	16/16	14/16
AG	None		10/10				9/10	
EG	None			8/10				8/10
Check			0/16	0/16			0/16	0/16

[a] First true leaf.
[b] The most recently expanded leaf. Symptomless at the time of attempted virus recovery.
[c] The numerator refers to the number of plants from which virus was recovered, the denominator to number of plants in the trial.

strain dominated in the plants that had an interval of 4 days or less between inoculations with the 2 vector-specific strains, regardless of which virus strain was administered first. No additional symptoms developed in about 30% of the plants that had received the AG strain 7 days or more after the EG strain. The majority of the plants inoculated with the EG strain and allowed to develop early symptoms before inoculation with the AG strain developed symptoms typical of the EG strain. This was interpreted not as partial interference, but as the result of rapid oat development in the greenhouse, giving the challenger virus little opportunity to affect disease development when introduced after a lengthy lag.

ACQUISITION, LATENT, AND INOCULATION FEEDING PERIODS.—Acquisition and inoculation feeding trials were conducted to determine more precisely the relations of the apple grain and English grain aphids to their vector-specific strains of the cereal yellow-dwarf virus. Individual aphids were transferred to the virus source plant (diseased Fulghum oats) and observed until the rostrum had been inserted into the plant tissue. The feeding period of the aphids was then considered to have started. Enforced fasts of 24 hours before acquisition feeding reduced the tendency of the apple grain aphid to move excessively when first transferred to the virus source plant. Both the aphid species thus began feeding almost immediately after transfer to the source plant.

A single acquisition feeding was used for each insect. The feeding periods used were: 1/6, 1/2, 1, 2, 4, 8, 16, 24, and 48 or 72 hours. Each test was replicated 3 times.

Following the acquisition period, individual aphids were transferred to healthy oat seedlings for serial inoculation feeding trials. The periods used were the same as in the acquisition study, with one aphid used for each series of serial transfers. Each aphid, upon removal from the virus source plant, was allowed to feed 10 minutes on a healthy seedling, then transferred to a second seedling and fed 30 minutes, transferred to a third seedling for 1 hr., and so on up to

TABLE 2.—*Acquisition, latent, and inoculation times, using the AG and EG vector-specific strains of the cereal yellow-dwarf virus, moving the aphids serially from plant to plant, left to right*

Acquisition feeding (hours)	Inoculation feeding periods[a] (hours)								
	1/6	1/2	1	2	4	8	16	24	72
Apple grain aphid									
16	0/18[b]	0/17	0/18	0/17	0/18	0/18	0/17	0/18	0/17
24	0/18	0/15	0/18	0/18	9/18	15/18	16/18	15/17	17/18
72	0/18	0/18	0/17	2/18	14/18	15/18	14/18	18/18	16/18
English grain aphid									
16	0/17	0/18	0/18	0/17	0/18	0/18	0/18	0/18	0/18
24	0/18	0/18	0/18	0/17	5/17	11/18	12/18	11/18	13/18
48	0/18	0/17	0/18	1/18	9/18	13/17	12/18	12/18	13/18

[a] Cumulative latent and inoculation time (hours): 1/6, 4/6, 1 1/6, 3 4/6, 7 4/6, 15 4/6, 31 4/6, 55 4/6, 127 4/6.
[b] The numerator refers to the number of plants from which virus was recovered, the denominator to number of plants in the trial.

TABLE 3.—*Transmission of the AG and EG strains of the barley yellow-dwarf virus by the instars of their respective vectors, the apple grain and English grain aphids*[a]

Instar	No. of plants	Transmissions
Apple grain aphid		
1	21	18
2	21	19
3	21	21
4	21	20
Adults	21	18
Nonviruliferous adults	15	0
English grain aphid		
1	30	24
2	30	20
3	30	27
4	30	23
Adults	30	26
Nonviruliferous adults	15	0

[a] Two insects given 24-hr. acquisition and inoculation feeding periods were placed on each seedling.

the final 72-hr. period.

Transmission did not occur with acquisition feeding of 16 hours or less. The data for the shorter acquisition periods are therefore omitted from Table 2.

The first transmissions, achieved after the vector was given a 24-hr. acquisition feeding period, followed serial transfers that allowed a latent period in the insect of 4–8 hours (Table 2). The longer acquisition feeding periods (48 or 72 hours) resulted in some transmission of virus (3 of 36 plants) after serial transfers allowing a latent period of only 100–220 minutes within the vector. Acquisition feeding periods longer than 24 hours tended both to reduce the latent period and to increase (slightly) the number of total transmissions in the serial transfers. Fasting the apple grain aphid before inoculation feeding did not lessen the latent period or increase the total number of transmissions.

The shortest acquisition period observed in this study was 24 hours for both AG and EG aphids. Longer acquisition periods tended to enable transmission in shorter inoculation feedings, but aphids that fed 24 hours on the source plant became well charged with virus, as shown by successive transmissions in the serial movements.

The transmission trials confirmed earlier work showing that the virus persists in the vector (1, 6, 11, 15). As long as the acquisition feeding period was 24 hours or more, transmission occurred in each feeding succeeding the 8-hr. inoculation feeding period. Each viruliferous vector transmitted virus to several successive plants with no source of added virus.

The time requirements of each aphid species were similar. Under similar conditions, the AG aphid brought about infection 151 times, the EG aphid, 112 times.

APHID INSTARS AS VECTORS.—Oswald and Houston (6) proved that the cereal yellow-dwarf virus is not passed from adult to progeny. This result has been confirmed by all who have used their method of obtaining virus-free aphids. Therefore, since nymphs are borne virus-free, a series of tests was made to determine the abilities of the various instars of the AG and EG aphids to act as vectors of the vector-specific strains.

The life cycle in the greenhouse permitted testing each instar. At 70°F each nymphal instar of both species occupied a stadium of at least 48 hours, making 24-hr. acquisition and inoculation feedings possible between molts.

Two virus-free nymphs of a given instar were placed on each source and receiver plant of a series. They were then removed by fumigation. Both species were handled similarly.

All instars of both species (Table 3) were effective vectors, both in acquisition and inoculation, of their respective strain of the barley yellow-dwarf virus. Adults likewise were vectors both in these and all reported trials of other workers. Aphids in any stage of development are potentially good vectors.

DISCUSSION.—Barley yellow dwarf, like most diseases, becomes increasingly complex as it is studied in greater detail. Allen (1) and Takeshita (11) extensively studied strain differences based on varietal reactions. Toko and Bruehl (13) and Rochow (9, 10) studied the occurrence of different strains based upon vector relationships. Bruehl and Toko (4) reported differences in host range on a species and genus level. The virus can now best be visualized as a complex of related entities varying in many ways.

The general occurrence of strains adapted to transmission by at least 2 aphid species in Washington should favor perpetuation of the virus, especially where aphid species populations may vary markedly from time to time (3). No data are available on the abundance of apple grain and English grain aphids, or of other species, from season to season or year to year. In the absence of such information it may be assumed that such changes in species populations have occurred and have selected against widespread occurrence of vector-specific strains such as the 2 described in this paper.

The lack of interference found in this study between the AG and EG strains, along with the absence of interference between strains either in the host or vector as found by Allen (1), makes it difficult to explain Allen's (1) failure to find mixtures in nature. The Dusty isolate of cereal yellow-dwarf virus shifted during greenhouse experimentation from non- to AG-specificity. The original strain studied in Washington tended to break up into an AG and an EG strain when subjected to repeated separation transmission by the 2 vectors (12). These observations indicate either the occasional occurrence of mixtures or the occurrence of unstable virus entities.

Studies of the barley yellow-dwarf virus indicate it to be a typical persistent virus (1, 6, 11, 15) apparently fitting Bennett's (2) Group 1 viruses in every respect. The results showed that the EG and AG strains required about 24 hours for acquisition, and several hours for efficient inoculation. No cases of

quick acquisition or inoculation, as obtained by Freitag (reported in Allen's (1) work), were seen. Watson and coworkers (15) stressed long acquisition and inoculation periods as essential to efficient transmission by *Rhopalosiphum padi* L.

These long feeding times are important, not only for experiment but in epidemiology. In a host-range study (4) it was noted that certain host grasses were inoculated with difficulty because the aphids, particularly the apple grain aphid, accepted many only when forced to feed on them under confinement. This work was with succulent seedlings.

Recovery of virus was difficult from many of the grasses past the seedling stage. This was interpreted to mean that in nature, where aphids may move freely from one grass to another, they would not remain on a host unpalatable to them long enough either to acquire or to transmit the virus. To this extent, host range studies may be misleading. It is probable that relatively few grasses serve as important virus reservoirs, because only a few may be sufficiently relished by aphids to multiply both aphid and virus.

The ability of all instars to act as vectors is advantageous to experimenters working with a minimum number of aphids.

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COMPARISON OF DEGREE AND DURATION OF SUSCEPTIBILITY OF BARLEY TO ERGOT AND TRUE LOOSE SMUT[1]

W. P. Campbell and L. E. Tyner[2]

SUMMARY

Twelve varieties of barley were grown in the greenhouse, and separate groups of plants were inoculated with loose smut and ergot on the day they first headed and at 2-day intervals thereafter for 10 days. Maximum susceptibility to *Claviceps purpurea* occurred as the plants first headed, and all were about equally susceptible at that time. Maximum susceptibility to *Ustilago nuda* occurred within 4 days of heading, but the varieties differed greatly in susceptibility. Susceptibility to either pathogen gradually decreased after heading, and in most cases was but slight after 10 days. The varieties that remained susceptible to ergot longest were those most susceptible in the field. Four varieties were almost completely free of infection by *U. nuda* following inoculation with the collection used at any time within 10 days after heading. Different factors apparently govern infection of barley by the 2 pathogens.

The ergot fungus *Claviceps purpurea* (Fr.) Tul. and the true loose smut fungus *Ustilago nuda* (Jens.) Rostr. invade the florets of barley at about the same time—during the flowering stage of the host. The spores of the fungi must enter the floral cavity to establish infection, and it appears that the plant is susceptible for only a relatively short period (3, 8). Differences in susceptibility of various varieties of cereals to ergot and loose smut have been observed and recorded (2, 3, 4, 6), but no comparative study has been made of the duration and degree of susceptibility of different varieties to the 2 pathogens. This paper reports on such a study of 12 common varieties of barley.

MATERIALS AND METHODS.—To screen barley in the field for susceptibility to ergot, 25 varieties were planted in randomized blocks, replicated 4 times and repeated a second summer. The plants were sprayed with an aqueous suspension of conidia of *C. purpurea* at 2-day intervals beginning at the time of earliest heading until well past the time of blossoming. The percentage of heads infected was the criterion of susceptibility. Twelve varieties, including the most and the least susceptible, were chosen for further study. Table 1 shows the average percentage infection of the varieties for the 2 years of the test.

These 12 varieties were then grown in the greenhouse, where their susceptibility to ergot and loose smut was compared. Collections of undetermined races of both pathogens were obtained for this purpose from the field in 1957. The inoculum of *C. purpurea* was prepared by diluting 1 ml of honeydew containing conidia in 100 ml of water, and that of *U. nuda* by suspending 0.2 g of spores in 100 ml of 0.002% aqueous solution of Aerosol OT (added to reduce surface tension, facilitating spore dispersal).

On the day the plants headed, 10 heads were inoculated with loose smut and 10 with ergot. The inoculum in each case was injected between the bracts into the floral cavity with a hypodermic syringe. This process was repeated with different plants every second day for 10 days.

When the plants were mature the percentage of florets infected with *C. purpurea* was recorded for each variety and for each time of inoculation. Seeds from the plants inoculated with *U. nuda* were grown in the field and the percentage of smutted plants that developed was recorded.

RESULTS.—*Ergot.*—The data from the greenhouse experiments are summarized in Table 1. The figures shown represent an average of 4 tests.

The varieties are listed in order of increasing susceptibility to ergot as determined by the field test, and the results of the greenhouse trials indicate why the varieties showed differences in the field. Those that were most susceptible in the field retained their susceptibility for the longest period in the greenhouse tests. In all varieties there was a gradual increase in resistance from the time of heading to the tenth day thereafter.

Loose smut.—Three separate plantings of the 12 barley varieties were made in the greenhouse in

TABLE 1.—*Per cent infection of florets of 12 varieties of barley inoculated with suspensions of Claviceps purpurea conidia at various intervals following heading*

Variety		No. of days after heading					
		0	2	4	6	8	10
Newal	(0.26)[a]	36	14	8	10	10	3
Husky	(0.48)	33	20	14	13	13	2
Gateway	(0.76)	40	27	24	14	11	3
Vantage	(1.43)	32	21	13	10	6	2
Olli	(2.32)	37	26	24	16	17	4
Peatland	(2.73)	27	18	16	16	7	3
Plush	(2.88)	40	26	29	18	9	3
Trebi	(6.40)	40	32	24	14	6	2
Velvon 11	(7.11)	38	36	29	28	30	8
Titan	(9.31)	43	32	26	22	14	8
Wolfe	(10.46)	40	36	19	25	13	11
Warrior	(17.14)	42	42	32	27	26	23

[a] Numbers in brackets after varietal names represent per cent of infected plants in field tests where the heads were sprayed with water suspensions of conidia at several 2-day intervals.

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TABLE 2.—*Per cent of loose smut infection in plants of 12 varieties of barley grown from seed inoculated with suspensions of Ustilago nuda conidia at various intervals after heading*

Variety	No. of days after heading					
	0	2	4	6	8	10
Newal	14	22	14	6	5	1
Husky	8	16	8	8	3	1
Gateway	20	26	21	10	2	0
Vantage	12	21	20	7	3	0
Olli	20	25	15	7	2	0
Peatland	11	11	14	5	2	1
Plush	9	8	10	5	6	0
Trebi	1	0	0	0	0	0
Velvon	0	0	0	0	0	0
Titan	0	0	0	0	0	0
Wolfe	14	26	41	32	13	3
Warrior	0	1	1	0	0	0

January and February, 1958. The harvested seed was planted in field plots. The data shown on percentage of smutted plants in Table 2 are based on observations of 3 plantings, each of which included several hundred plants. Data from the 3 plantings were in close agreement.

The number of plants that produced heads was considerably less than the number of seeds planted. Seed produced in the greenhouse at the latitude of Edmonton during the winter tends to be small in size. Some damage to the embryos may also have resulted from inoculation by needle. However, it is unlikely that the relative performance of varieties was affected by this factor since the per cent of stand was roughly equivalent in all cases.

Several observations may be made from considering the data. The varieties Trebi, Titan, Velvon, and Warrior showed little if any infection after inoculation on any date. Susceptibility to infection was greatest during the first 4 days after heading, and resistance was essentially complete by the tenth day in all 12 varieties. The relative susceptibility of the varieties (except, possibly, Wolfe) to loose smut did not correspond to their susceptibility to ergot.

DISCUSSION.—The 12 varieties show one factor common to infection by the 2 fungi: susceptibility was reduced to a minimum on the tenth day after heading. Anthesis is probably complete in all varieties within 4 days after heading, maximum susceptibility apparently occurs before fertilization, and resistance is rapidly attained after fertilization is effected.

The varieties differed widely in susceptibility to loose smut, and less widely in susceptibility to ergot. Four varieties were almost completely resistant to loose smut at any date of inoculation, but no variety was similarly resistant to ergot. The same 4 varieties were among the most susceptible to ergot, and it thus appears that susceptibility to *U. nuda* in barley is based on factors different from those involved in susceptibility to *C. purpurea*.

Campbell (1) has shown that infection by *C. purpurea* results from penetration of the ovary wall by mycelium arising from spores within the floral cavity, that the mycelium ramifies intercellularly through the tissues of the wall, and that the pathogen may even sporulate before the embryo is attacked and colonization becomes intracellular. Pedersen (5) offered evidence that *U. nuda* does not infect the ovary by way of the stigma but by invasion from the floral cavity directly through the ovary wall, intracellularly to the integuments, and thence to the embryo. The decrease in susceptibility to infection is thus probably independent of physiological changes that may occur within the style and stigma after passage of the pollen tube. However, physiological activities undoubtedly change throughout all tissues of the young seed following fertilization. Such changes may impose restrictions on infection in different ways for different pathogens. Growth also rapidly accelerates after fertilization, and mechanical barriers to infection may be established quickly (7).

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EFFECTS OF INOCULATIONS WITH LEPTOGRAPHIUM SPP. ON WESTERN WHITE PINE[1]

Charles D. Leaphart and Lake S. Gill[2]

SUMMARY

Seven isolates of *Leptographium* were used in several series of inoculations on healthy western white pine trees. About 2300 inoculations, involving different techniques of wounding and several types of inoculum, were made on different portions of the roots and stems of 321 trees during a 3-year period. All isolates caused some degree of cambial necrosis, but differences in virulence were observed. Definite callusing developed around the edges of nearly all lesions within 1 year of inoculations; fewer than 3% of the positive inoculations were still active 3 years later. The study suggests that: 1) the potential of this fungus to cause damage in healthy white pine trees is inversely proportionate to the tree's ability to callus diseased tissue; 2) the fungus contributes to the deterioration of the structural root systems of pole-blight-affected stands; and 3) the virulence of the form of *Leptographium* present in a diseased tree may be associated with both the lesion development pattern on the tree and that tree's rate of decline.

Between 1949 and 1951 a series of inoculations on western white pine (*Pinus monticola* Dougl.) were made with *Leptographium* spp., a fungus commonly associated with stem lesions characteristic of the pole blight disease (1, 5). A preliminary report on the condition of inoculations after 1 year was published in 1951 (2). In 1954 the inoculation project was closed since by then it was evident from other studies (6) that the stem lesions were probably an effect rather than a basic cause of the decline and death of trees suffering from the pole blight disease. More recent studies (4) suggest that pole blight probably develops as a result of soil conditions that lead to a deterioration of the root system and subsequent decline of the entire tree. Furthermore, independent inoculation studies by Hubert (3) and Parker (8) led them to conclude after 2 and 4 years of observations, respectively, that *Leptographium* was pathogenic to a degree but probably not the cause of pole blight.

The findings reported here agree with those of Hubert and Parker, but the longer period of observation (3–5 years) and the greater number of inoculations have provided additional information on *Leptographium* as a pathogen. None of the inoculated trees developed pole blight symptoms, so the sole purpose here is to record briefly the behavior of a fungus with pathogenic potentialities. In a preliminary report (2), mention was made of limited inoculations with *Armillaria mellea* (Vahl.) Quel. and *Poria weirii* Murr. All inoculations with these fungi were negative or complicated by contamination at the close of the project, and hence warrant no further comment.

SCOPE OF THE EXPERIMENT.—Nine inoculation areas were established in northern Idaho and northeastern Washington: 7 in healthy stands of white pine poles of ages most likely to develop pole blight (40–100 years); 1 in a 15-year-old plantation; and 1 in a 175-year-old stand. All were in disease-free stands occurring in the portion of the white pine type affected by pole blight.

Only dominant or codominant trees were inoculated, because these classes most frequently contract the disease in nature. In some trees, up to 4/5 of the major lateral roots were girdled or cut before inoculation in an attempt to reduce vigor and predispose the trees to disease. This treatment did not fulfill its purpose. Host vigor remained apparently unchanged and the inoculations were comparable to those on trees that had not been root pruned.

Inoculation techniques varied from no wounding to removing a slice of bark 2 in. long. One to several bark punctures, made with a dissecting needle or sharp scalpel, were most frequently used since they had proved adequate for obtaining infection with least disturbance of the pine tissues.

Inoculations were placed in tops, mid-boles, root collars, buttress roots, and lateral roots, either singly or in combination on a single tree. The number of inoculations at a particular area on a tree was varied from a single puncture or group of punctures to circular or spiral series of sufficient frequency to ensure girdling if inoculations were successful. Several trees were killed in this manner, but the symptoms attendant on their death were not suggestive of pole blight.

The inoculum most commonly used was a mass culture of the organism on malt agar medium. Inoculum was smeared on the prepared area with a sterile scalpel and covered with a moist sterile dressing of cotton held in place with a wrapper of Cellophane. Infection was also obtained with 2 other techniques: 1) binding cotton containing a water spore suspension of the organism over wounds or punctures, and 2) binding pieces of wood supporting a culture of the organism to roots, the majority of which were wounded. The soil was immediately replaced over all root inoculations.

About 2300 inoculations were made on 321 trees over a 3-year period. Fourteen of these trees were

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subjected to various degrees of root pruning before inoculation. Seventy-four check trees were treated with sterile media inoculations, involving all types and degrees of wounding and nearly all portions of the tree.

RESULTS.—Seven isolates of *Leptographium*, selected by a screening process previously described (2), were used for the inoculations. Table 1 summarizes the measurements of a representative sample of these inoculations examined in detail at the close of the project in 1954. Of the 1023 root or root collar and 426 stem inoculations in this sample, 98.6 and 94.5%, respectively, were positive. Of the 570 controls examined, fewer than 3% (on the stems) and 7% (on the roots) showed some degree of arrested necrosis, indicative of contamination.

By 1954 most of the inoculations were inactive and the lesions were callusing (Fig. 1) or had completely callused over. Fig. 2 shows the typical external symptoms of a positive inoculation on a stem. Pitch-soaked soil wads developed around positive inoculations on roots. The extent and girdling action of lesions on the stem of a 15-year-old tree are represented in Fig. 3, which also shows callus tissue forming on the outer margins of the two coalesced lesions.

Much variation was observed in lesion development on the above-ground portion of the tree. The longest lesion, 36 in., was caused by isolate 602 on a lower bole inoculation (16 ft. above ground). The average length of all lesions on lower boles was 6 in., and only isolates 602 and 845 caused lesions that on the average exceeded this, almost doubling the average length of lesion for all isolates combined. Stem inoculations near the tree tops and at the ground line were not as aggressive, apparently because annual radial growth in these regions is normally greater than on the lower bole. *Leptographium* thus appears to invade woody tissue more rapidly where growth is slow.

Of 941 inoculated roots, 43 were killed. Although isolates 602, 603-2, 819, and 850 accounted for about 85% of the mortality, every isolate was involved in the death of at least one root. The size of killed roots varied from 0.25 in. to 1.5 in. in diameter. Only 19 of the root inoculations appeared to be active in 1954. As indicated by the presence of mycelial fans, 14 were invaded by *Armillaria mellea*, 6 on the roots of 1 tree. The other 5 showed no evidence of the callusing typical of arrested lesions. Two were on roots that had been killed, and necrosis was slowly progressing toward the root collar. The cause of the progressive necrosis in these 5 roots was not determined.

The largest root lesion was 28.0 in. long and 1.0 in. wide and was caused by isolate 763. This isolate varied greatly in its ability to produce damaging lesions; the average lesion corresponded very closely to the average of all isolates combined. Both 763 and 603-2 were used in all 3 years of inoculation studies. Table 1 points out what appears to be an attenuation in culture since the lesions from the 1951 work were nearly half as large as those from the 1949 and 1950 inoculations. As was observed in the stem inoculations, isolate 602 produced lesions that averaged almost twice the size of the average lesion for all isolates combined. The lesion variation encountered in root inoculations is exemplified by the samples in Fig. 4. Examples of aggressiveness are represented by the root on the left (positive) and the root on the right (negative) of the figure.

As previously pointed out, the degree of wounding had no significant effect on the success of infection by any particular isolate, but wounds or punctures were essential to the success of stem inoculations. Of 84 roots inoculated without wounding, 16 were positive, 7 of which could be attributed to faulty technique: placing inoculum near wounds made in excavating

TABLE 1.—*Summary of Leptographium and control inoculations examined in detail from 1949 to 1954*

Isolate and year of inoculation[a]	Portion of tree inoculated							
	Upper bole		Lower bole		Root collar		Roots	
	% positive[b]	Lesion length[c]	% positive	Lesion length	% positive	Lesion length	% positive	Lesion length
603-2 (1949 and 1950)	100.0	1	100.0	3	[d]		98.4	4
603-2 (1951)			94.6	2	100.0	1	96.3	2
763 (1949 and 1950)	100.0	3	100.0	4			99.5	3
763 (1951)			90.3	2	64.7	1	93.2	1
602 (1951)			100.0	6	100.0	4	100.0	7
811 (1951)			100.0	3	20.0	4	100.0	3
819 (1951)			100.0	3	15.4	2	86.1	2
845 (1951)			100.0	6	100.0	1	89.5	2
850 (1951)			100.0	3			100.0	4
Avg. all isolates	100.0	2	98.2	4	74.4	2	96.9	3
Controls	0.0	0	2.6	4			6.5	2

[a] Only isolates 603-2 and 763 were used in 1949–1950. These have been separated from the 1951 inoculations in order to show what appears to be a difference in virulence in the same isolate due possibly to attenuation in cultures.
[b] The percentage of total inoculations that developed necrotic tissue subsequent to treatment.
[c] Widths of lesions varied from only a trace of necrosis to 2 in. wide. The rating, therefore, is based on average length of lesion, in inches: 0 = no lesion; 1 = trace to 1.9; 2 = 2.0–3.9; 3 = 4.0–5.9; 4 = 6.0–7.9; 5 = 8.0–9.9; 6 = 10.0–11.9; 7 = 12.0–13.9.
[d] No inoculations attempted where spaces are blank.

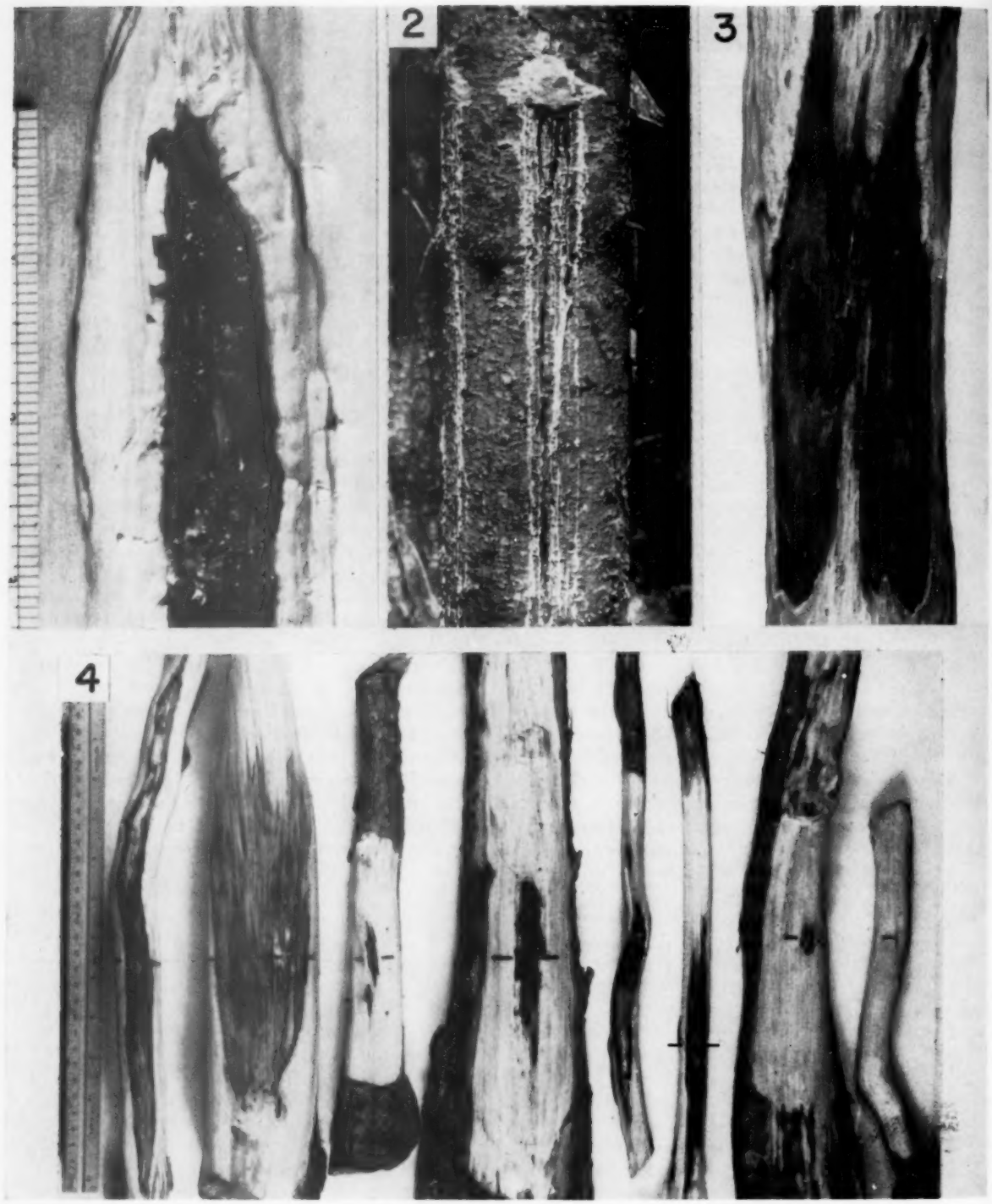

Fig.1–4. Inoculations with *Leptographium* spp. on western white pine trees.—**Fig. 1.** Surface view of a partial callus on a lesion 2 years following a stem inoculation. The pencilled line outside the callus shows approximately the original limits of the lesion.—**Fig. 2.** Typical resin flow from a positive stem inoculation made in 1949 on an 85-year-old tree.—**Fig. 3.** Two positive inoculations on the stem of a 15-year-old tree collected 1 year following treatment. Note coalescence of the 2 lesions and the usual callusing near their tips as observed in a majority of inoculations examined.—**Fig. 4.** A representative sample of roots inoculated in 1951 and collected in 1954. The bark has been peeled from the lesions and surrounding wood surface, as it was in Fig. 3, to illustrate lesion development. The inoculation point was at the horizontal black line on each root.

the roots for treatment. The remaining 9 resulted from inoculations with infected root sections placed on undisturbed root surfaces. Only 9 such inoculations were made; 2 were still active 6 years after inoculation. Under favorable natural conditions, unwounded white pine roots can presumably become infected with *Leptographium* if enough inoculum of the right type comes in contact with them.

DISCUSSION.—Obvious differences in virulence of the isolates were established despite the variation in technique of wounding and the use of different inocula. The authors (6) have observed that some trees in the final stages of decline from pole blight show relatively little stem necrosis, while the cambium on others may be extensively killed. This study suggests that more virulent forms of *Leptographium* might account for the extensive cambial necrosis observed in the latter class of trees. The authors and other people interested in this problem have also observed a variation in rate of disease progress within individual trees, i.e., from a healthy to an advanced pole blight condition. The observations suggest that the virulence of the particular form of *Leptographium* within each tree may contribute to the variation in individual tree decline.

The study showed that *Leptographium* is not a persistent canker-forming agent on vigorous, healthy dominant and codominant trees, but reveals nothing on its behavior in hosts of low vigor. Nevertheless, it is commonly associated with cambial necrosis that develops on diseased trees. These studies also suggest that the potential of this fungus for damage is inversely proportionate to the ability of the tree to callus; younger trees and faster growing portions of older trees particularly showed aggressive callusing of the lesions.

Table 1 shows that the fungus has a considerable capacity for damage to structural roots. This, coupled with the fact that *Leptographium* lesions are common on roots ¼ in. and larger in diameter in pole blight areas, suggests that it is involved in the deterioration of the root systems of pole-blight-affected stands, a condition associated with the disease (4, 7). No attempts have been made to determine the role that *Leptographium* may play in the death of fine roots. Nevertheless, in white pine root systems occupying the upper 1 ft. of soil in 60- to 80-year-old stands, high rootlet mortality and low rootlet density are both significantly correlated with pole blight incidence (4). Soils with low available moisture storage capacities and shallow effective depths generally support stands having high rootlet mortality and low rootlet density. To date, pole blight has been found only on soils with low available moisture storage capacities and generally where shallow effective soil depths occur. The ecology of *Leptographium* spp. and its pathogenicity, especially of fine roots, merit further investigations under various environmental conditions.

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EFFECTS OF GAMMA RADIATION ON BROWN ROT AND RHIZOPUS ROT OF PEACHES AND THE CAUSAL ORGANISMS[1]

Louis Beraha, G. B. Ramsey, M. A. Smith, and W. R. Wright[2]

SUMMARY

Maximum levels of gamma radiation that permitted conidial germination, colony formation, and mycelium development of *Rhizopus nigricans* and *Monilinia fructicola* in Czapek's and Tochinai's media were dependent on the organism, the suspending medium, and the phase in development of the fungus. Artificially inoculated peaches kept at 80–85°F were free of Rhizopus rot for at least 10 days after a dose of at least 250,000 rep.[6] A dose of 200,000 rep inhibited brown rot for 10 days at 80–85°F. Unirradiated peaches were completely rotted within 5 days. Textural and skin-color abnormalities (softening and skin browning) were noted following irradiation at 400,000 rep or higher, but not at 300,000 rep.

Estimates of losses from decay of peaches in New York City retail stores have been conservatively set at 13–24 per cent.[3] The ubiquitous causal agents, *Rhizopus nigricans* Fr. and *Monilinia fructicola* (Wint.) Honey, possibly account for all the decay recorded. Low-temperature storage and chemical surface disinfectants offer only partial decay control. The success of gamma radiation in extending the shelf life of such fresh fruits as oranges and lemons[4] made it desirable to investigate the use of this energy source for controlling peach rots and their causal agents. A preliminary report of these investigations has been made.[5]

MATERIALS AND METHODS.—The materials and general techniques used in preparing and handling the fungi, *R. nigricans* and *M. fructicola*, were similar to those previously reported for *Penicillium digitatum* and *P. italicum*.[4]

Firm-ripe Elberta and Red Haven peaches were used in 2 separate experiments. After being selected for size and absence of bruises and decay, the peaches were washed 5 minutes in a solution containing 2500 ppm of free chlorine, rinsed in tap water, and allowed to dry. Wounds were made by puncturing opposite sides of each fruit 5 mm deep with 7 pins circularly arranged and spaced 5–7 mm apart. Following a momentary dip into a sterile distilled water suspension containing 100,000–500,000 viable spores per ml of either *R. nigricans* or *M. fructicola*, the fruits were kept in humidity chambers for 20 hours before irradiation. Peaches inoculated with *R. nigricans* were kept separate from those inoculated with *M. fructicola*. For each experiment, a replicate consisted of 2 peaches inoculated with either *R. nigricans* or *M. fructicola*, placed in a small perforated polyethylene bag, and sealed in a No. 2 can. For each of two experiments, a dose with a control was replicated 4 times, giving a total of 8 replications. Immediately before and after irradiation, samples were kept refrigerated until returned to the laboratory. After irradiation, the lids of the cans were cut off in the laboratory and replaced loosely to permit ventilation. Temperature ranges were 80–85°F during the incubation period. Control samples were similarly prepared, taken to the irradiation site, held at room temperature during the longest irradiation time, incubated, and observed periodically along with the irradiated samples. Degree of infection was assessed by arbitrary infection grades: 0 = no infection; 1 = less than ¼ of fruit rotted; 2 = ¼–½ of fruit rotted; 3 = ½–¾ of fruit rotted; 4 = ¾–entire fruit rotted; 5 = fruit completely rotted. Along with the numerical gradation of severity, notes were made when decay was first visible and of its progress during the holding period. Injury was determined visually by comparison with unirradiated and inoculated controls.

All fruits and culture samples in No. 2 cans were rotated at 2 rpm in urns adjacent to cooling upright fuel elements at the Argonne National Laboratory, Lemont, Ill. Unless otherwise stated, all doses are approximate, to within 10%.

RESULTS.—*Studies on spores.*—The radio-sensitivity of conidia of *R. nigricans* and *M. fructicola* in Czapek's and Tochinai's media as determined by germination is given in Fig. 1. The maximum level of irradiation that allowed germination of conidia of *R. nigricans* was about 500,000 rep.[6] At this dose germination

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[6] For roentgen-equivalent-physical, the amount of ionizing radiation that would deliver 93 ergs to a gram of wet tissue.

TABLE 1.—*Colony growth*[a] *resulting from irradiated conidia and irradiated hyphae of Rhizopus nigricans and Monilinia fructicola suspended in Tochinai's (T) and Czapek's (C) media*

Dose (rep)	Medium	*R. nigricans*		*M. fructicola*	
		Conidia	Hyphae	Conidia	Hyphae
1,000,000	T	0	0	0	0
	C	0	0	0	0
500,000	T	0	0	0	0
	C	G	0	0	0
400,000	T	D	0	0	0
	C	G	D	0	0
300,000	T	G	D	0	0
	C	G	D	0	0
250,000	T	G	D	0	0
	C	G	G	0	0
200,000	T	G	D	D	D
	C	G	G	0	0
150,000	T	G	G	G	D
	C	G	G	D	0
100,000	T	G	G	G	D
	C	G	G	D	D
50,000	T	G	G	G	G
	C	G	G	G	G
0 (control)	T	G	G	G	G
	C	G	G	G	G

[a] D = growth delayed; G = normal growth; 0 = no growth.

was not appreciably better in either Tochinai's or Czapek's medium. *M. fructicola* showed about 50% germination after irradiation of conidia suspended in Tochinai's medium at 1,000,000 rep (the highest dose tested), but did not germinate in Czapek's medium at this dose. Similarly, germination was better in Tochinai's medium than in Czapek's medium at all doses above 100,000 rep. Germ-tube growth was inversely proportional to the dose received (Fig. 2). As has been demonstrated with *P. digitatum* and *P. italicum*, germination and germ tube growth were proportional to the radiation dose, i.e. shortened distorted tubes were evident after relatively high doses, whereas near

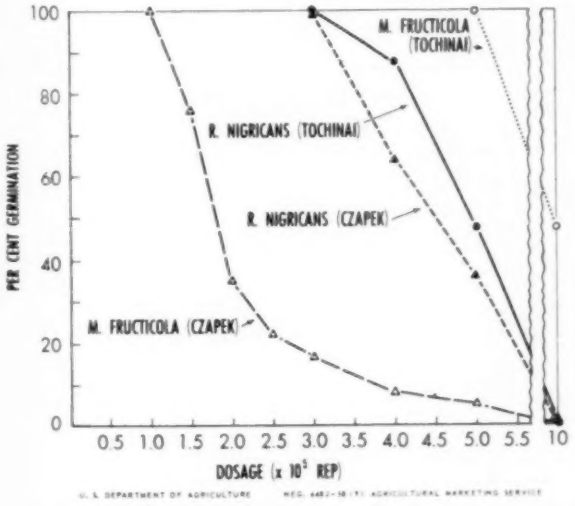

Fig. 1. Germination of *Rhizopus nigricans* and *Monilinia fructicola* conidia in Tochinai's and Czapek's media 20 hours after gamma irradiation.

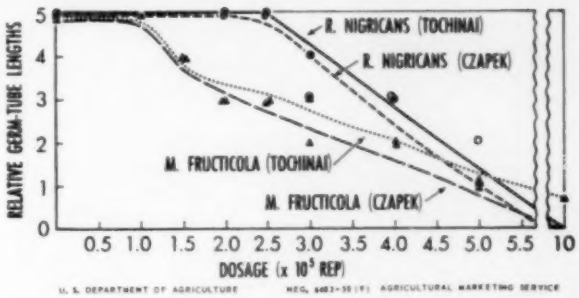

Fig. 2. Relative germ-tube lengths of *Rhizopus nigricans* and *Monilinia fructicola* conidia in Tochinai's and Czapek's media 20 hours after gamma irradiation.

normal size tubes developed after lower doses.[4] This was again observed with *Rhizopus nigricans* in both Tochinai's and Czapek's media and with *Monilinia fructicola* in Czapek's medium. However, germination of *M. fructicola* suspended in Tochinai's medium compared with corresponding germ-tube growth (Fig. 1, 2) demonstrates that at doses allowing 100% germination (500,000 and 150,000 rep), respective germ tubes were 1/5 and 4/5 the size of control tubes. Hence, germination and germ-tube growth, apparently, may function independently after irradiation of viable conidia under some conditions.

Studies on mycelium.—1) *Influence of radiation dose and suspending medium on colony formation.*—At the intervals tested, the maximum irradiation level that allowed survival of conidia (as determined by continued development to form colonies) was about 500,000 rep for *R. nigricans* and 200,000 rep for *M. fructicola* (Table 1). Survival was also modified by the nature of the suspending medium. Growth of *R. nigricans* following irradiation of conidia was typical at 500,000 rep in Czapek's medium, but only at 400,000 rep in Tochinai's medium. Conversely, growth of *M. fructicola* following irradiation of conidia was typical at about 200,000 rep in Tochinai's medium, but only at 150,000 rep (the next-lowest dose tested) in Czapek's medium.

2) *Influence of radiation dose and suspending medium on survival of mycelium.*—Table 1 gives the data for survival of young hyphae of *R. nigricans* and *M. fructicola* following irradiation. Again, both the dose and the nature of the suspending medium influenced survival. The maximum level of irradiation that allowed resumption of mycelial growth of *R. nigricans* was about 400,000 rep. At this dose, growth resumed when mycelium had been grown on Czapek's medium but not when grown on Tochinai's medium. On the other hand, mycelium of *M. fructicola* grew following doses of about 200,000 rep or lower when previously grown on Tochinai's medium. On Czapek's medium, no growth took place above a dose of about 100,000 rep. As we have demonstrated for *Penicillium italicum*, levels of radiation that may curtail (a) germination, (b) subsequent development of conidia to form colonies, and (c) continual growth of estab-

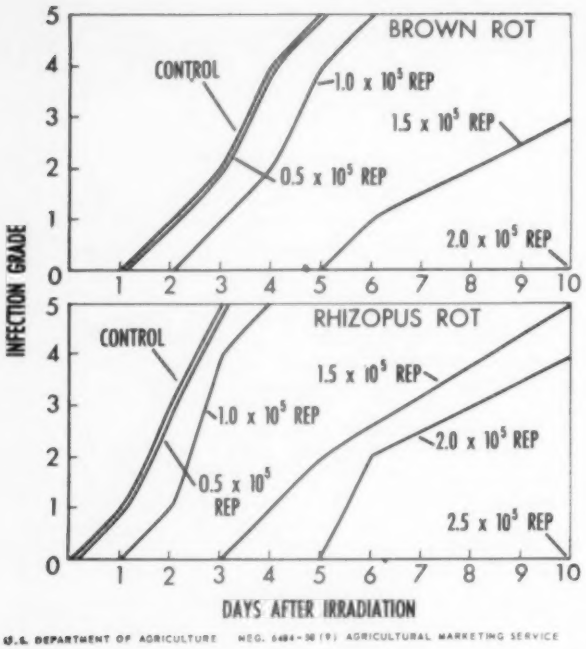

Fig. 3. Development of brown rot *(M. fructicola)* and Rhizopus rot *(R. nigricans)* at 80–85°F following gamma irradiation of artificially inoculated Red Haven and Elberta peaches.

lished hyphae will differ for each of these different phases of fungal development.[4]

Studies with fruit.—Essentially no differences were noted between the two varieties of peaches tested. A comparison of the effects of radiation on brown rot and Rhizopus rot of peach, demonstrates dissimilar reactions of two different fungi in the same suscept. Peaches inoculated with *M. fructicola* and stored at 80–85°F remained free of infection for 10 days following irradiation of at least 200,000 rep. At 150,000 rep, infection was delayed 6 days, at which time it was noticed on 1 replicate out of 8; at 10 days only 2 replicates were infected. Average infection ratings of decayed peaches showed a reduced rate of rotting with increases in dose. Thus, control replicates were completely rotted within 5 days, but peaches receiving 100,000 rep were rotted in 6 days; after 150,000 rep, only a moderate amount of rot was evident at 10 days (Fig. 3).

A dose of at least 250,000 rep was required to control *R. nigricans* completely. At 200,000 rep, the next-lower dose tested, infection was delayed 6 days, at which time 3 replicates out of 8 showed infection. At 10 days, 5 replicates showed infection. As with brown rot, proportionally more decay was evident in a shorter period with decreases in the dose. The controls and those receiving 50,000 rep were completely rotted within 3 days; after 100,000 rep, within 4 days; after 150,000 rep, 6–10 days; and after 200,000 rep, with those showing infection, peaches were completely rotted at 10 days (Fig. 3).

Textural and skin-color abnormalities in the form of softening and excessive browning (cinnamon to ochraceous brown) were noted following irradiation at 400,000 rep, but not at 300,000 rep. Hence, for the dose intervals tested, the minimal doses that control brown rot (150,000–200,000 rep) and Rhizopus rot (200,000–250,000 rep) are within the limits that do not cause visibile injury to peaches (for the period of the experiment).

Discussion.—Our studies with citrus decay indicated that the balance between the radiation doses necessary to control rot and those that injure the fruit is precarious.[4] Peaches, on the other hand, apparently can withstand doses higher than necessary to suppress or control brown rot or Rhizopus rot. Naturally, in the case of peaches and citrus, injury was based on a visual approximation of degree and kind. Conceivably, injury may also take the form of cellular breakdown or damage to tissue constituents in addition to visual rind color and textural changes. The levels of radiation at which injury may be visible or at which decay organisms are affected, however, will differ among commodities. Some of the influencing features of the commodity will be water content and the presence of proteinaceous and other components. The variation in dose ranges for complete kill of different fungi in peaches and some of the factors responsible for the variation have been reported. It may be concluded that a safe dose of gamma radiation must be established for each decay-producing organism and each commodity.

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DEVELOPMENT OF PRATYLENCHUS PENETRANS AND TYLENCHORHYNCHUS MARTINI ON RED CLOVER AND ALFALFA[1]

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SUMMARY

The growth of Kenland red clover *(Trifolium pratense)* and Atlantic alfalfa *(Medicago sativa)* was significantly reduced by *Pratylenchus penetrans*, either alone or in combination with *Tylenchorhynchus martini*, in greenhouse experiments. *P. penetrans* reproduced readily in both plants. *T. martini* reproduced readily on red clover, but poorly on alfalfa. Neither plant was adversely affected by *T. martini*. With a 1:1 combination of the 2 nematodes the growth of both plants was reduced the same amount as with *P. penetrans* alone. *P. penetrans* increased as well in the presence of *T. martini* as it did when alone, but *T. martini* in the presence of *P. penetrans* increased only 10–25% as much as it did when alone.

Species of root-lesion (*Pratylenchus*) and stylet (*Tylenchorhynchus*) nematodes, along with other plant-parasitic nematodes, are frequently found in fields of red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.). Previous study showed that a mixture of species of *Pratylenchus* caused severe injury to these plants under greenhouse conditions (1). An investigation was conducted to determine the relative effect of *P. penetrans* (Cobb, 1917), *T. martini* Fielding, 1956, and a combination of the two on the growth of red clover and alfalfa.

MATERIALS AND METHODS.—Root-lesion and stylet nematodes used for inoculum were from colonies reared, respectively, on corn (*Zea mays* L.) and sorghum (*Holcus sp.* L.). Both colonies were started with collections from a field of red clover. The nematodes were apportioned by diluting suspensions in water.

One-half-gallon glazed crocks were filled to within 2 in. of the top with soil heated 5 minutes with steam to 80–85°C. Fifty-ml suspensions containing nematodes were poured over the surface of the soil. Control crocks received 50 ml of tap water. In addition, all crocks received 50 ml of liquid from nematode suspensions that had been virtually freed of nematodes by being passed several times through a U.S. No. 325 sieve. The inoculum was covered with 2 in. of steamed soil, and certified seed of Kenland red clover and Atlantic alfalfa was sown on the surface. The seeds were covered with a thin layer of steamed soil. The filled crocks contained about 1800 g of soil (dry-weight basis). Each crock received a suspension of commercial nodule-producing bacteria when it was first watered. The crocks were arranged in 4×4 Latin squares on a greenhouse bench.

When plants were about 3 weeks old the stands were thinned to 5 plants per crock by clipping off excess plants below the soil surface. At intervals during the experiments the top growth was cut back to 2 in. and the harvested material was dried and weighed. At the conclusion of the experiments the plants were cut off at the surface of the soil, and the tops were dried and weighed. The roots were carefully freed from the soil and washed, blotted, and weighed.

The number of nematodes in roots was determined by hanging the roots in a mist chamber and collecting as long as nematodes emerged. The number of nematodes in soil was determined by using a modified Seinhorst inverted-flask technique (2). Two 300-g aliquots were processed from each crock.

The experiment was repeated once, with essential agreement in results. The data reported herein are

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The investigation reported is in connection with a project of the Kentucky Agricultural Experiment Station, and is published with the approval of the Director.
[2] Plant Pathologist.

TABLE 1.—*Mean dry weight (g per crock) of top growth of Kenland red clover and Atlantic alfalfa growing in soil initially infested with Tylenchorhynchus martini or Pratylenchus penetrans or both*

Harvest number	Age (days)	Red clover				Alfalfa			
		Control	T[1]	P[2]	T + P	Control	T	P	T + P
1	136	3.0	3.4	2.8	3.1	2.5	2.6	2.9	2.7
2	152	1.4	1.6	1.2	1.5	1.5	1.6	1.6	1.6
3	174	1.7	2.1**	1.0**	1.0**	1.6	1.9**	0.9**	1.1**
4	204	2.3	2.3	0.6**	1.0**	1.5	1.5	0.6**	0.7**
5	227	1.7	1.7	0.4**	0.5**	0.8	1.0	0.4**	0.4**
6	256	1.2	1.2	0.4**	0.4**	1.5	1.4	0.4**	0.4**
7[3]	267	2.9	2.9	1.4**	1.6**	3.5	3.8	2.2**	2.3**
Total		14.2	15.2	7.8**	9.1**	12.9	13.8	9.0**	9.2**

[1] *T. martini.*
[2] *P. penetrans.*
[3] Includes stubble.
** Difference from control significant at the 1% level.

TABLE 2.—*Numbers of nematodes in soil and roots of Kenland red clover growing in soil infested with Tylenchorhynchus martini or Pratylenchus penetrans or both*

	Control	*T. martini*	*P. penetrans*	*T. martini* + *P. penetrans*
Nematodes per 100 g dry soil	2	5,290	8,056	529 + 8,953
Nematodes per 1 g roots	<1	12	4,387	0 + 3,085
Nematodes per crock	40	96,672	170,014	9,522 + 177,196

from the second experiment, in which the numbers of nematodes placed in the crocks in the different treatments were: control, 0; *Pratylenchus*, 900; *Tylenchorhynchus*, 900; and *Pratylenchus* plus *Tylenchorhynchus*, 450 of each.

RESULTS.—The yields of top growth are set forth in Table 1. Top growth was significantly decreased for both species of plants growing in soil infested with *P. pentrans*. This was similar to results obtained previously with a mixture of species of *Pratylenchus* (1). Top growth was not reduced for either species of plant growing in soil infested solely with *T. martini;* in fact, top growth significantly increased in 1 harvest.

Root weight was significantly lower for both species of plants growing in soil infested with *P. penetrans*. The respective mean fresh weights, in g per crock, of roots of red clover were 12.3, 12.1, 5.7, and 5.2 for plants growing in soil infested with no nematodes, *T. martini* alone, *P. penetrans* alone, and *T. martini* plus *P. penetrans*, respectively. With alfalfa the respective mean weights of roots were: 22.7, 21.4, 16.8, and 17.1 g per crock. Root weight was unaffected by *T. martini* alone.

In crocks containing red clover, *P. penetrans* increased about 189 times when the soil was initially infested with 900 nematodes, and about 394 times when the soil was initially infested with 450 along with 450 of *T. martini* (Table 2). In crocks containing alfalfa the respective increases were about 110 and 143 (Table 3).

In crocks containing red clover, *T. martini* increased about 107 times when the soil was initially infested with 900 nematodes, and about 21 times when the soil was initially infested with 450 along with 450 of *P. penetrans* (Table 2). In crocks containing alfalfa the respective increases were about 11 and 6 times (Table 3).

DISCUSSION.—The combination of nematodes had the same effect on growth of the plants as the root-lesion nematode alone. The fact that the combination inoculum contained one-half the number of root-lesion nematodes in the *P. penetrans* inoculum does not necessarily indicate an adverse effect of the stylet nematode on plant growth. It has been shown previously that doubling the number of root-lesion nematodes in the inoculum does not produce a much greater effect (1).

The factors of increase stated above are based on the terminal nematode population and may not be maximal. On this basis it is evident that the activity of *P. penetrans* had an adverse effect on the development of the population of *T. martini*. Root damage caused by the endoparasitic root-lesion nematode seriously reduced the food supply of the ectoparasitic stylet nematode. On the basis of the number of stylet nematodes per g of roots (number of stylet nematodes per crock divided by wt. of roots per crock) red clover supported 7989 in crocks containing *T. martini* alone, and 1831 in crocks containing the mixture of nematodes. In the same order, alfalfa supported 482 and 184 stylet nematodes per g of roots. Other antagonistic effects may also have been involved.

Whether the activity of *T. martini* affected the development of the population of *P. penetrans* is not evident. Whether there was an adverse effect cannot be determined. At first glance, a stimulatory effect seems likely. However, when the data for *P. penetrans* are expressed as above for *T. martini*, the results are inconsistent. Red clover supported 29,825 nematodes per g of roots in crocks containing *P. penetrans* alone and 34,076 *P. penetrans* per g of roots in crocks containing the mixture of nematodes. On the other hand, alfalfa supported, in the same order, 5887 and 3761 *P. penetrans* per g of roots. The differences between these figures are not significant, whereas those between the numbers of *T. martini* are significant. Also, smaller initial populations of nematodes have frequently been reported to have higher rates of increase than larger initial populations (3, 4, 5), and it is possible that *T. martini* had little or no effect on *P. penetrans*.

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TABLE 3.—*Numbers of nematodes in soil and roots of Atlantic alfalfa growing in soil infested with Tylenchorhynchus martini or Pratylenchus penetrans or both*

	Control	*T. martini*	*P. penetrans*	*T. martini* + *P. penetrans*
Nematodes per 100 g dry soil	<1	561	4,846	141 + 3,180
Nematodes per 1 g roots	<1	10	695	0 + 414
Nematodes per crock	15	10,312	98,904	2,538 + 64,319

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RHIZOPUS DECAY OF SWEET POTATOES AS AFFECTED BY CHILLING, RECURING, AND HYDROWARMING AFTER STORAGE[1]

T. T. McClure[2]

SUMMARY

Sweet potatoes developed less Rhizopus decay if recured at 85°F on removal from storage than if recured at lower temperatures or not recured. Two days of recuring at 85° was as effective as 4 or 8 days, indicating the feasibility of recuring during transit. Immersion of roots in 110° water for 15 minutes raised their temperature from 60° to 85° without increasing decay. Hydrowarming seems to be a safe and rapid method of heating roots for recuring during transit. Chilled sweet potato roots developed more Rhizopus decay than those not chilled. Such decay was affected by the chilling temperature, the duration of chilling, and the recuring temperature. Chilling 1 day at 32°F or 4 days at 45° increased decay when the roots were placed for recuring at 65° or 75°, but not when they were recured at 85°. The minimum time for effective recuring of chilled roots was the same as that for unchilled roots.

Rhizopus decay of sweet potatoes is one of their major post-harvest decays, which destroy an estimated 18.8% of the crop during and after transit to market (8). Stored sweet potatoes are usually washed and packed in preparation for shipment to market. This handling accidentally wounds many roots, making them subject to Rhizopus decay. Recuring roots before shipment has been suggested as a possible method of reducing decay (1, 2). Laboratory tests were made during 1956 and 1957 to determine whether injured roots could be satisfactorily recured during a simulated transit period to avoid delay in marketing. The most pertinent questions were 1) what temperature caused adequate recuring, 2) how long a recuring period is necessary, and 3) how may roots be warmed rapidly to a recuring temperature?

Sweet potatoes marketed during the winter may be exposed to short periods of low temperature during or after transit. Sweet potatoes are known to be injured by prolonged exposure to temperatures below 55° or 60°F (3, 5, 7). Laboratory tests were made to determine the effect of short periods of low temperature on the development of Rhizopus decay of sweet potato roots.

METHODS.—In 1956 Nemagold sweet potatoes obtained from the field at harvest were cured for 10 days at 85°F in the laboratory and stored at 55° until used. From time to time, roots were removed from storage, some for immediate inoculation and others for further storage at 45° or 32° for 1, 2, 4, or 8 days before inoculation. Roots were inoculated by halving lengthwise and dipping immediately in a suspension of spores of a *Rhizopus* sp. in beef-peptone broth. The concentration of spores was about 100 per low-power field of a microscope. Inoculated roots were placed in 4-qt. baskets at 85°, 75°, 65°, or 55° for 1, 2, 4, or 8 days to test the effects of these conditions on recuring.

Humidity was kept high by draping wet towels over the baskets and covering them with polyethylene film. After the various recuring periods, all roots were transferred to 65°F, covered with wet towels, and held 8 days to permit development of incipient decay. Decayed roots were removed every 2 days, and the number decayed by the end of the final 65° holding period was totaled. The amount of decay was used as a measure of effectiveness of recuring.

In 1957 Orange Jersey sweet potatoes were similarly cured and stored at 60°F until used. The methods were the same as in 1956 except that some roots were hydrowarmed to about 85° by immersing 15 minutes in 110° water before wounding and inoculation.

Each test lot consisted of 10 random halves of roots. The experiment with Nemagold sweet potatoes was done 8 times, and that with Orange Jersey was done 10 times.

RESULTS.—The average decay of sweet potato roots was markedly less after recuring at 85°F than after recuring at 75° (Tables 1 and 2). This difference occurred even when the roots were chilled at 32° or 45° before recuring (Tables 2 and 3).

The effectiveness of recuring, as measured by the amount of decay, was essentially the same whether roots were recured for 2, 4, or 8 days. The amount of decay following recuring at 85°F for 1 day was intermediate between that of roots recured for 2 days and that of checks held at 65°.

Roots can be warmed from 60°F to about 85° in 15 minutes by immersion in 110° water. Hydrowarming did not result in more decay of roots after recuring than that of similar roots not hydrowarmed (Table 2).

Sweet potatoes chilled 4 days at 45°F before wound-

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TABLE 1.—*Per cent of Nemagold sweet potato roots with Rhizopus decay after being removed from storage at 55°F., inoculated and recured immediately at various temperatures for 1–8 days, and then held 8 days at 65°*

Recuring temperature (°F)	Days at recuring temperature				
	1	2	4	8	Average
85	21.3	10.0	6.3	12.5	12.5
75	42.5	28.8	30.0	27.5	32.2
65	51.3	38.8	38.8	33.8	40.6
55	47.5	56.3	47.5	50.0	50.3
LSD for recured roots				16.2(5%)	21.4(1%)
LSD for averages				8.1(5%)	10.7(1%)

ing and inoculation and recured 8 days at 75° or 65° decayed more than comparable unchilled roots (Table 3). Roots chilled at 45° for 8 days did not decay significantly more than unchilled roots when the recuring temperature was 85°. Decay was increased by exposure of roots to 32° for only 1 day followed by recuring at 75° or 65°. An exposure of 8 days at 32° was needed to increase decay of roots if they were subsequently recured at 85°. Roots chilled at 32° for 4 days were effectively recured at 85° in 2 days, the same time that was needed for unchilled roots (Table 2).

DISCUSSION AND CONCLUSIONS.—During the digging season, sweet potatoes may be marketed immediately after harvesting. They start their transit period at field temperatures, often above 80°F, and they usually encounter mild weather in transit. Such roots have been reported to develop less Rhizopus rot 1 week after arrival than cured and stored roots (4). When cured roots are removed from storage, fresh wounds are produced by the necessary handling. Wounded roots maintained at transit temperature of 55° to 60° decay excessively when later warmed to 65°, an approximate average retail temperature. Recuring of roots is indicated to control Rhizopus decay, and the most convenient time for recuring is during transit. Maintaining recuring temperatures at this time would also eliminate the possibility of chilling injury to roots in transit during winter months.

TABLE 2.—*Per cent of hydrowarmed or chilled Orange Jersey sweet potato roots with Rhizopus decay after being recured for 1–4 days at 85° or 75°F and then held 8 days at 65°*

Treatment and recuring temperature (°F)	Not recured[a] (check)	Days at recuring temperature				Av. decay of recured roots[b]
		1	2	3	4	
No treatment						
85°	52	37	28*	24*	23*	28
75°		58	51	52	63	56
Hydrowarmed						
85°	47	39	19*	18*	11*	22
75°		37	45	53	48	46
Chilled 4 days at 32°						
85°	66	45	29**	31**	32**	34
75°		65	50	55	65	59

[a] Roots not recured were held at 65° for 8 days.
[b] LSD for averages: 11.0 (5%) and 14.6 (1%).
* and ** indicate significant differences from check at 5 and 1% levels, respectively.

The present study shows that Rhizopus decay of sweet potatoes after removal from storage may be effectively reduced by recuring the roots 2 days at 85°F. Holding roots 1 day at 85° would reduce decay less effectively. These results indicate that recuring roots at 85° during transit should reduce Rhizopus decay at the retail level. Full utilization of the transit time as a recuring period would require preheating the roots to 85°, because of the time required to bring sweet potatoes from a storage temperature of about 60° to a recuring temperature of 85°. This may be done rapidly, without harming the roots, by hydrowarming them 15 minutes in water at 110°. Washing and hydrowarming might perhaps be combined in a single operation.

Increased decay in storage has been reported as a symptom of chilling injury of sweet potatoes (5, 6, 7). This symptom may occur during prolonged holding at low temperatures or when removed to higher temperatures after a brief chilling period (5). There is little agreement on the duration of chilling temperatures necessary to increase decay of roots. Lauritzen (5) found that 29 days at 40° or 32°F was needed to

TABLE 3.—*Per cent of Nemagold sweet potato roots with Rhizopus decay after being chilled at 45° or 32°F for 1–8 days and recured at various temperatures for 8 days plus an additional 8 days at 65°*

Treatment and recuring temperature (°F)	Not chilled (check)	Days at chilling temperature				Av. decay of chilled roots[a]
		1	2	4	8	
Chilled at 45°						
85°	12.5	10.0	6.3	13.8	18.8	12.2
75°	27.5	41.3	42.5	50.0*	52.5**	46.6
65°	33.8	43.8	48.8	62.5**	61.3**	51.4
Chilled at 32°						
85°	12.5	16.3	16.3	13.8	37.5*	21.0
75°	27.5	55.0**	61.3**	57.5**	61.3**	58.8
65°	33.8	62.5**	61.3**	67.5**	71.3**	65.7

[a] LSD for averages, 45° chilling: 9.2 (5%) and 12.2 (1%)LSD for averages, 32° chilling: 10.3 (5%) and 13.7 (1%).
* and ** indicate significant differences from checks at 5 and 1% levels, respectively.

increase decay of Yellow Jersey sweet potatoes. Increased decay of roots was reported to occur in the Porto Rico variety after chilling 10 days at 40° or 4 days at 32° (7).

The amount of decay of chilled sweet potatoes has been shown by the present study to be affected by the subsequent recuring temperature. When the recuring temperature was 75° or 65°F, roots exposed 4 days to 40° or 1 day to 32° decayed more than did unchilled roots. Eight days at 45° or 4 days at 32° were tolerated without a significant increase in decay when the recuring temperature was 85°. Since retail store temperatures average about 65°, a temperature that favors decay development, shippers of sweet potatoes should avoid even a few days of accidental chilling during winter transit to market. Recuring temperatures during transit should protect roots from chilling injury and thus should reduce Rhizopus decay at the retail level.

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ENTOMOSPORIUM LEAF SPOT OF PHOTINIA GLABRA AND PHOTINIA SERRULATA[1]

P. D. Stathis and A. G. Plakidas[2]

SUMMARY

Leaf blight of pear (*Pyrus* spp.) and loquat (*Eriobotrya japonica*), caused by *Entomosporium maculatum*, is a common and serious disease of these hosts in Louisiana. An *Entomosporium*, indistinguishable morphologically from that on pear, quince, and loquat, was recently found to cause severe spotting and defoliation of *Photinia glabra* and *P. serrulata* in nurseries in southeast Louisiana. The disease appears to be new for these two species of *Photinia*. *Entomosporium* isolates from 5 different hosts—pear, quince, loquat, *P. glabra* and *P. serrulata*—were morphologically alike but differed in pathogenicity and in colony size on artificial media. The pear and quince isolates were similar; they were strongly pathogenic to pear and loquat, nonpathogenic to *P. glabra* and *P. serrulata*, and formed small colonies. The isolates from *P. glabra* and *P. serrulata* formed a second distinct group; they were strongly pathogenic to the 2 *Photinia* species, pear, and loquat, and their colonies were statistically significantly larger than those of the pear and quince isolates. The isolates from loquat constituted a third group; they were strongly pathogenic to pear and loquat, weakly pathogenic to *P. glabra* and *P. serrulata*, and formed colonies intermediate in size between those of the other 2 groups. Each group is considered to represent a distinct physiologic race of the fungus. Observational and experimental evidence indicates that the photinia race did not arise from the pear and quince race. It is conjectured that the race pathogenic to photinias arose from the loquat race, probably by mutation.

In the spring of 1957 *Photinia glabra* (Thumb.) Max. plantings in 2 nurseries in southeast Louisiana were severely affected by a leaf spotting and defoliating disease. Scattered *P. serrulata* Lindl. bushes were also affected by the disease. In the spring of 1958 the disease reappeared on the same hosts, somewhat more widespread on *P. serrulata* than in the previous year. An *Entomosporium*, morphologically indistinguishable from *E. maculatum* Lév. (*Fabraea maculata* (Lév.) Atk.), the cause of leaf blight of pear and quince, was found associated with the disease of photinias. The photinia disease appeared to be new. No reference to an Entomosporium disease on these two species of *Photinia* was found in pathological literature, although such a disease is known on *P. arbutifolia* (Ait.) Lindl. in California (9).

Entomosporium leaf spot is common and severe on pears in Louisiana, scattered plantings of which

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occur near the nurseries where the disease was found on photnias. The same disease occurs on loquat (*Eriobotrya japonica* Lindl.) grown extensively in the same nurseries. Quince (*Cydonia oblonga* Mill.) is not grown in the area where the disease was found on photinias, but a number of quince seedlings in an experimental orchard of the Department of Horticulture of Louisiana State University, Baton Rouge, were found very heavily affected. There is also one report of Entomosporium leaf spot on pyracantha in Louisiana (7). A preliminary report has been published on finding the disease on *P. glabra* for the first time (8).

Since the disease appeared to be new on photinias, the probability was considered that inoculum for these hosts might have originated from either pear or loquat. Observation indicated rather strongly that infection of photinias did not come from pear. *P. glabra* bushes growing several years directly under

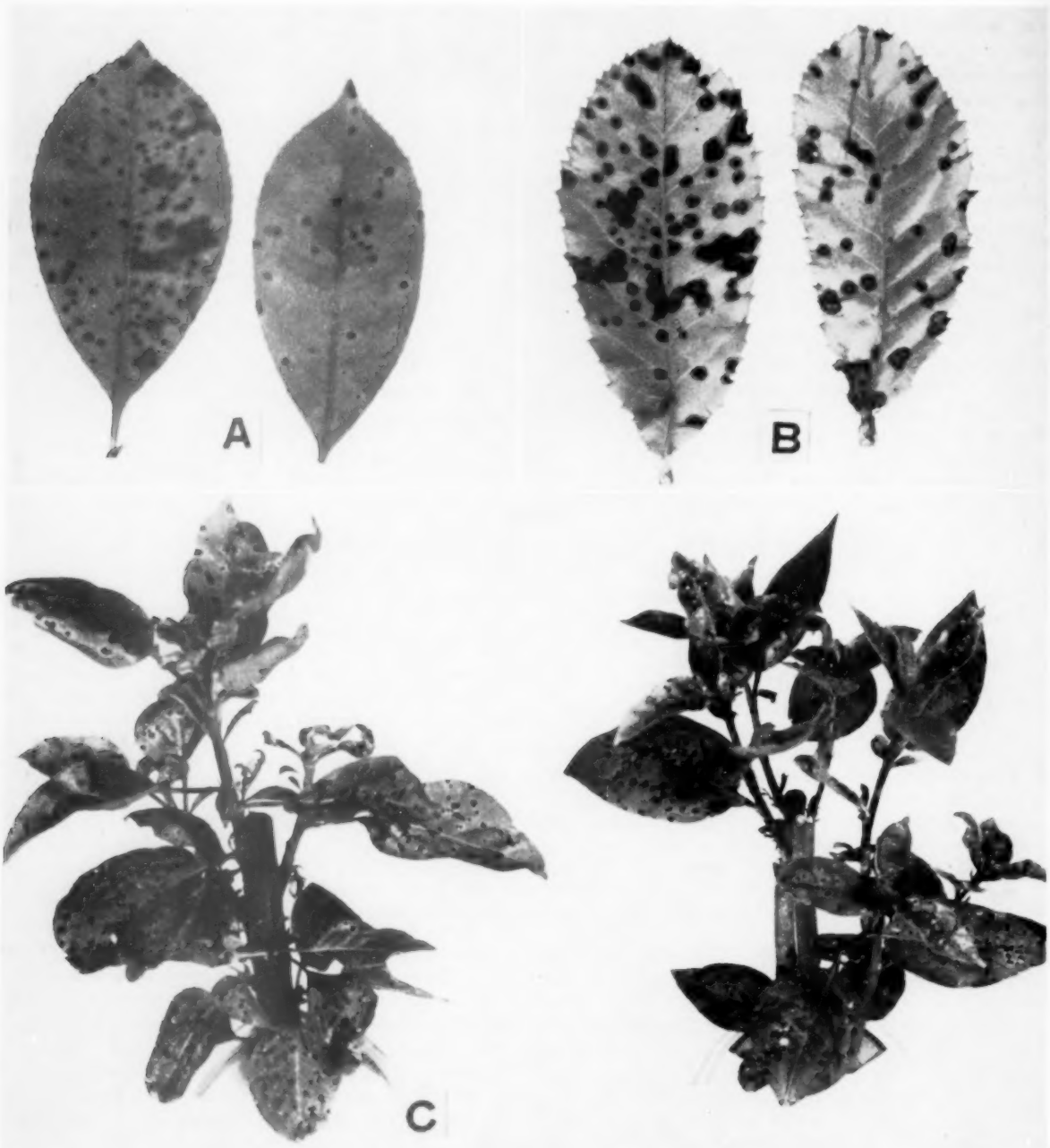

Fig. 1. Typical symptoms on *Photinia glabra* **(A)** and *P. serrulata* **(B)**. Infection of pear seedlings with pure culture isolates of Entomosporium from *P. glabra* **(C)** and pear **(D)**.

severely diseased pear trees were completely free of infection. On the other hand, observation tended to incriminate the loquat as source of inoculum. In the nursery in which the disease was first found, *P. glabra* plants about 200 feet from heavily infected loquats were severely diseased, whereas *P. glabra* plants about 1000 feet from the infected loquats showed only a trace of infection. This study was undertaken to determine the host relationship of *Entomosporiums* occurring on different hosts in Louisiana and to ascertain the source of inoculum for the infection of photinias.

Klebahn (3), after morphological and physiological studies of *Entomosporium* from various hosts, recognized 2 species: *E. thuemenii* (Cooke) Sacc., on *Crataegus;* and *E. maculatum*, on pear, loquat, quince, and *Amelanchier*. Fungus taken from pear infected pear, but gave uncertain results on quince and did not infect medlar (*Mespilus*), *Cotoneaster*, or *Crataegus*. Fungus taken from quince, on the other hand, infected quince and pear readily, caused slight infection on *Mespilus*, *Amelanchier*, and *Cotoneaster*, and did not infect *Crataegus*. Using morphological characters as criteria, Grove (2) recognized *Entomosporium maculatum* as the only existing species, with 3 varieties: a) var. *domesticum*, on *Mespilus;* b) var. *mespili*, on *Cotoneaster*, quince, *Pyrus sylvestris*, *Mespilus*, and *Amelanchier;* and c) var. *cydoniae*, on quince. Stewart (11) was able to infect pear with the fungus from *Crataegus* and quince, quince with the fungus from *Crataegus* and pear, and *Crataegus* with the fungus from pear. Laubert (4) was of the opinion that the forms of *E. maculatum* on pear and quince were morphologically and physiologically different, and suggested that they be respectively differentiated into f. *piri* and f. *cydoniae*. He also reported that the fungus from pear would infect apple leaves, but that the closely related forms from *Mespilus*, *Cotoneaster*, and *Crataegus* did not appear to infect fruit trees. Laubert's view was challenged by Wenzl (12), who stated that in Austria the transfer of the fungus from quince to pear was very common. Moore (5) suggested that it is best to regard the fungi occurring on the different hosts, except possibly that on *Crataegus*, as strains of the species *E. maculatum*. According to Smith (9), *E. maculatum* is one of several fungi causing leaf spots of *Photinia arbutifolia* in California. However, he was of the opinion that the fungus on this host may not be identical with that causing leaf blight of pear, since the disease is not known on pears in California.

Description of disease.—Disease symptoms are similar on both species of *Photinia* except that, in general, the leaf spots are more numerous and somewhat larger on *P. serrulata* than on *P. glabra*. Very young infections appear as minute, slightly raised dots on either surface of the leaf. These dots gradually enlarge, reaching a diameter of about 3–4 mm and extend to the opposite surface (Fig. 1-A,B). Older spots have a slightly depressed center and raised, definite margins, and are more or less circular. On the juvenile, red-colored foliage of *P. glabra* the young, non-necrotic, lesions are a darker purple than the surrounding healthy tissue, whereas the older, necrotic, spots have ashy-gray centers and dark-purple margins. On the older, green growth, the young lesions are purplish, and the older, necrotic lesions have ashy-gray centers and dark-purple margins surrounded by an indefinite, lighter-purplish halo. The centers of the necrotic spots on both surfaces are dotted with minute black specks, the acervuli of the fungus. The spots are discrete in light infections, but in heavy infections often coalesce to form large lesions of irregular shapes. Infections are mostly limited to the leaf blades, and only occasionally a few spots may occur in petioles and tender young shoots. Heavily infected leaves fall prematurely, resulting in severe defoliation.

Infection starts in the spring, on the foliage of branches close to the ground, and spreads gradually upward. This coincides with the profuse production of conidia on fallen overwintered leaves, indicating that the overwintered leaves serve as the chief source of inoculum for primary infection.

Materials and methods.—Two methods were used to obtain pure cultures of the fungus: tissue plating and single-spore isolations. In the first method, infected leaves were washed with a brush under running water and surface-disinfected with either mercuric chloride or calcium hypochlorite; then, small pieces of tissue from the margin of the lesions were planted on PDA (potato-dextrose agar) in Petri dishes, 5 pieces per plate. After 1 week at room temperature, the plates were examined and only tissue pieces showing neither fungus nor bacterial growth were transferred separately to autoclaved beanpods in tubes. This was done because *Entomosporium* is known to grow extremely slowly, and any growth appearing within a week of plating was certain to be a contaminant. After a second incubation period, of about 15 days, small, compact, glistening, white fungus growths became visible on some of the tissue pieces on the beanpods. These were transferred to agar slants.

Monoconidial isolates were obtained as follows: Under a dissecting microscope, conidial masses were picked from acervuli with a sharp needle and suspended in a small volume of sterile water. A few drops of the conidial suspension were poured into Petri plates containing 2% water agar. The plates were rotated to spread the suspension evenly on the surface of the agar and left uncovered for a few minutes to allow the water of the suspension to evaporate. Then, under the dissecting microscope, single conidia were lifted with the needle and transferred to beanpod agar slants. This method, found to be much easier and quicker than the tissue-plating method, was the one most commonly used in this study.

Potted plants of the following hosts were used for the inoculation experiments: *Photinia glabra*, *P. serrulata*, pear (*Pyrus* spp.), loquat (*Eriobotrya japoni-*

ca), and a *Pyracantha* sp. Healthy plants of quince (*Cydonia oblonga*) were not available. The plants of *P. glabra* and pyracantha were grown from cuttings; those of the other 3 hosts from seed. The bulk of the pear seedlings were raised from seed of a single tree, a Chinese sandpear, variety Pineapple, but a few came from seed of unknown parentage. However, variability among seedlings was not a problem, for all the pear seedlings tested proved highly susceptible to infection.

Comparative growth studies of isolates from different hosts were made on 4 different solid media—potato-dextrose, carrot-dextrose, malt extract, and beanpod-dextrose agars. Because the fungus sporulated much more abundantly on beanpod-dextrose agar, only that medium was used for routine transfers and for the production of inoculum. It was prepared as follows: 200 g of either fresh or frozen string beans were steamed in the autoclave for 30 minutes in 500 ml of water, macerated in a Waring Blendor, and filtered through 4 layers of cheesecloth. Water was then added to bring the volume to 1000 ml, and 20 g of dextrose and 17 g of agar were added. After the agar was melted, the medium was tubed and sterilized 20 minutes at 15 lb. pressure.

In all inoculation experiments, inoculum consisted of a conidial suspension in tap water adjusted to a density of about 25 conidia per low-power field of the compound microscope. In most instances the inoculum was applied under low pressure with an Erway glass sprayer connected to a compressed air line, but in some instance a hand-operated atomizer was used to apply the inoculum. The inoculated plants were kept in a large moist chamber for 5 days after inoculation, and then placed on a bench in the greenhouse. The greenhouse temperature was kept constant at about 80°F.

Results.—In an exploratory cross-inoculation experiment, 2 plants each of pear, loquat, *Photinia glabra*, and *P. serrulata* were separately inoculated with a mixture of isolates from 5 hosts—pear, quince, loquat, *P. glabra*, and *P. serrulata*. Results were clear-cut. The pear and quince isolates infected pear and loquat, but infected neither *P. glabra* nor *P. serrulata*. The isolates from both species of photinia infected all 4 hosts. The loquat isolates caused heavy infection on pear and loquat, and very light infection (a few scattered lesions) on *P. glabra* and *P. serrulata*.

One isolate from each host was picked at random and used for all the main cross-inoculation experiments and also for the morphological, cultural, and physiological comparisons of the different isolates. These isolates were: Ac from *P. glabra*, Hc from *P. serrulata*, Dc from pear, Gt from quince, and Cc from loquat.

The results of 5 separate experiments, 2 plants of each host in each experiment, are summarized in Table 1. The results of cross-inoculations with single isolates were identical with those obtained from inoculations with mixtures of isolates. The pear and quince isolates infected pear and loquat readily, but failed to infect the photinias. The isolates from both species of photinia readily infected *P. glabra*, *P. serrulata*, pear (Fig. 1-C), and loquat.

The loquat isolate produced heavy infection on pear and loquat, but gave erratic result on photinias; infections resulted in some of the tests but not in others. Also, in some instances, *P. glabra* became infected but not *P. serrulata*, or vice versa, and in every instance the infection was light (few small spots). None of the isolates was pathogenic to pyracantha.

Reisolations were made from pear and loquat seedlings that had been inoculated and infected with isolates from *P. glabra* and *P. serrulata*, and these reisolates were used to inoculate both species of photinia, and pear and loquat along with plants inoculated with the original isolates from these hosts. The pathogenicity of the original isolates was not affected by passage through a different host. Thus, after passage through pear and loquat, the photinia *Entomosporium* behaved exactly like the original isolations; it readily infected its original hosts, *P. glabra* and *P. serrulata*, and also pear and loquat. Furthermore, the resisolates were indistinguishable in cultural characters from the original photinia isolates. The photinia form of the fungus, whatever its origin, has become a stable physiologic race.

The results of cross-inoculations showed clearly that the photinia *Entomosporium* is pathogenically distinct from the forms on pear, quince, and loquat. The same 5 isolates used in cross-inoculations were compared morphologically and culturally to determine whether there were any differences that would correlate with pathogenicity.

Fifty conidia from each isolate from 20-day-old cultures on beanpod-dextrose agar were measured. Following are the average sizes for each isolate: pear, 22.8×13.6 μ; quince, 22.0×12.6 μ; loquat, 21.9×12.9 μ; *P. glabra*, 24.5×15.6 μ; *P. serrulata*, 22.0×12.6 μ. Statistical analysis showed that the differences were not significant. Measurements of conidia formed on the leaves of the 5 host plants also showed no significant differences in size, shape, color, or septation.

Table 1.—*Summary of the results of cross-inoculations between 5 Entomosporium isolates and 5 hosts (two plants of each host in each experiment)*

	Isolate and its source				
Inoculated	Ac *P. glabra*	Hc *P. serrulata*	Dc Pear	Gt Quince	Cc Loquat
Pear	+[a]	+	+	+	+
Loquat	+	+	+	+	+
P. glabra	+	+	−	−	±
P. serulata	+	+	−	−	±
Pyracantha	−	−	−	−	−

[a] + = infection; − = no infection; ± = erratic results—infection in some trials, none in others.

TABLE 2.—*Average diameter (mm) of 35-day-old colonies of Entomosporium isolates from 5 different hosts on PDA at various temperatures*

Isolate from	Temperature (°C)					
	5	10	15	20	25	30
P. glabra	0	2.9[a]	6.2	7.0	8.5	0
P. serrulata	0	2.8	6.5	7.8	9.2	0
Pear	0	1.4	4.1	4.4	4.6	0
Quince	0	1.1	2.4	4.3	4.8	0
Loquat	0	1.7	4.9	5.6	6.3	0

LSD .05 = 1.13; LSD .01 = 1.50

[a] Means of 5 replications.

It was noted early in this study that rate of growth was consistently greater for the isolates from both species of photinia than for the isolates from the other hosts. In a series of experiments the growth of isolates from the 5 hosts was compared on: a) 4 different media (potato-dextrose, carrot-dextrose, bean-pod-dextrose, and malt-extract agars) at a constant temperature of 20±1°C. b) a single medium (PDA) buffered at different pH levels at a constant temperature of 20±1°C; c) a single medium (PDA) at various temperatures (5–30°C). The figures in Table 2 are representative of the findings of the series of cultural studies. None of the isolates grew at 5°C or 30°C. The optimum temperature for all isolates appeared to be 20–25°C. The same relationship was found when the isolates were compared on 4 different media at a constant temperature of 20±1°C, and at different pH levels on buffered PDA. In all instances, the growth of the photinia isolates was statistically significantly greater than that of the pear and quince isolates. In general, the growth of the loquat isolates was intermediate between that of the photinia and the pear and quince isolates, but the differences were not always significant.

DISCUSSION.—The Louisiana photinia *Entomosporium* is morphologically indistinguishable from *E. maculatum*, and therefore cannot be considered as a new species. However, it fits well the concept of "physiologic race," as defined by Ainsworth and Bisby (1) and Stakman and Harrar (10), because it differs from the type (the fungus of pear and quince) in both pathogenicity and growth characters. On the basis of differences in pathogenicity and growth characters, the isolates from the 5 different hosts used in this study are classified in the following 3 groups, which are considered to represent 3 distinct physiologic races: group 1, pear and quince isolates strongly pathogenic to pear and loquat, nonpathogenic to *P. glabra* and *P. serrulata*, and forming small colonies on artificial media; group 2, isolates from *P. glabra* and *P. serrulata* strongly pathogenic to these two species of photinia, pear, and loquat, and forming colonies on artificial media significantly larger than those of the isolates of group 1; group 3, loquat isolates strongly pathogenic to pear and loquat, weakly pathogenic to *P. glabra* and *P. serrulata*, and forming colonies in culture intermediate in size between those of groups 1 and 2.

No satisfactory explanation can be offered regarding the origin of the race pathogenic to photinias. However, since no way of introduction can be traced, it is not unreasonable to speculate that it arose from pre-existing races on some other host, probably either pear or loquat. This leads to a second speculation: the manner of origin of this race. Physiologic races in other groups of fungi, and notably in rusts, have been shown to arise chiefly by hybridization, and to a smaller degree by mutation. A requisite for hybridization is the existence of a sexual stage. The *Fabraea* (sexual) stage of this fungus has never been found in Louisiana. In 1941 Plakidas (6) reported that he had failed to find the perfect stage of the fungus on overwintered infected pear leaves, and, in the course of this study, a diligent but fruitless search for the *Fabraea* stage was made on overwintered infected leaves of pear, loquat, and photinia. It appears then that the perfect stage of this fungus does not occur at all in Louisiana, or occurs so rarely as to be easily overlooked. In either case, it does not seem very likely that the photinia race arose from hybridization. It is conjectured that it arose as a mutant from some pre-existing race, probably from that on loquat.

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CUCURBIT MOSAIC VIRUSES IN CALIFORNIA[1]

R. G. Grogan, D. H. Hall, and K. A. Kimble[2]

SUMMARY

The most prevalent viruses infecting cucurbits in the desert valleys of southern California during 1956–1958 were strains of WMV (watermelon mosaic virus). Second in prevalence were strains of cucumber mosaic virus. Both are spread by aphids, and are rarely if ever transmitted by seed of commercial varieties and species of cucurbits. Squash mosaic virus, although commonly transmitted by seed of commercial varieties and species of cucurbits, was extremely rare in the desert valleys because spread was precluded by the paucity of its cucumber beetle vectors. In areas of the state where cucumber beetles occur more abundantly, squash mosaic virus was more prevalent. Strains of WMV were reisolated following mechanical inoculation of several varieties and species of plants in the families *Umbelliferae*, *Malvaceae*, and *Leguminosae*. Strains of the virus were isolated from alfalfa, *Malva parviflora*, and *Melilotus indica* collected in nature, indicating that these, and possibly other hosts, may be important as natural sources of primary WMV inoculum.

The literature on naturally occurring mosaic viruses and virus diseases of cucurbit hosts is replete with various names for both the viruses and the diseases. In many instances, the viruses are incompletely described and characterized, engendering considerable uncertainty and confusion on their identity and relationships, and the etiology of the diseases. Some reports have been concerned with seed transmission, insect vectors, or host range of a virus, but have seldom presented the information on all three that is so essential for understanding the etiology of the diseases.

The present report is concerned with the identity, relative prevalence, and etiology of cucurbit mosaic viruses in California, with special emphasis on the viruses of cantaloupe, watermelon, and squash in the desert valleys of Southern California.

REVIEW OF LITERATURE.—*Occurrence of cucurbit mosaic viruses in California.*—During the past several years, cucurbit crops (cantaloupe, watermelon, Honey Dew melon, and squash) grown in the Imperial Valley and other desert areas of Southern California for spring and early summer harvest, have been severely damaged by mosaic virus diseases. In the authors' experience, only rarely are plants infected early in the growing season, but in late May and June practically 100% of the plants usually become infected. That has been the situation since 1940 or earlier. In that year Milbrath (18) reported that a mosaic disease was widespread in many fields of watermelon grown in the Imperial Valley for spring harvest. No mention was made of mosaic infection of cantaloupe, squash, or other cucurbits, undoubtedly being grown in the area at that time.

A report in 1942 by Middleton and Whitaker (16) was concerned primarily with a "lethal disease" of cantaloupe caused by a strain of CMV (cucumber mosaic virus), but it mentioned "Common Cantaloupe Mosaic" and said it was a virus disease that had been prevalent in the Imperial Valley for many years. The virus was not identified, but was said to be seed-borne in cantaloupe; however, data were not presented on percentage of seed transmission.

In 1946 Middleton and Whitaker (17) reported widespread occurrence of "cantaloupe mosaic," presumably the same as the "Common Cantaloupe Mosaic" previously mentioned. They reported that yields of cantaloupes and Honey Dew melons were decreased, with Honey Dews the more severely affected. They tested several Honey Dew and cantaloupe seed stocks used to plant commercial acreage that year, and reported virus seed-transmission by all. They inferred that infected seed was the source of the cantaloupe mosaic virus in Imperial Valley, but presented no evidence that the seed-borne virus was the same as that common in the cantaloupe fields. In 1949 Middleton (15) reported data from surveys of the Imperial and Palo Verde valleys, in southern California, to determine the prevalence of viruses infecting cantaloupes and other cucurbits in the two areas. Samples of virus-infected plant material were collected from 53 local areas in the period May 9 to June 13, 1949. *Cucurbita pepo*, *Citrullus vulgaris*, and *Vigna sinensis* were inoculated with sap from each sample, and the viruses were separated on the basis of reaction on these three hosts into 3 groups: 1) "Cucumber virus 1," which caused symptoms on all 3 hosts; 2) "an undescribed cucumber virus," which caused symptoms only on squash; and 3) "squash mosaic virus," which caused systemic symptoms only on squash and watermelon. "Squash mosaic virus" was most prevalent, being present in 160 of 179 collections. The "undescribed cucumber mosaic virus" was present in 13 collections, and "cucumber virus 1" in 6. The present authors interpret the above data as follows: The virus that Middleton called Cucumber virus 1 is the same as Cucumber mosaic virus in this paper; the virus that he called squash mosaic virus should be called watermelon mosaic. This conclusion is supported by Freitag's report that watermelon did not react to squash mosaic virus inoculations (9), and by the report of Lindberg, Hall, and Walker (12) that 7 of their collections of squash mosaic virus showed no reaction on watermelon, and that 1 strain produced local lesions but no systemic reaction.

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Some of the virus collections called "undescribed cucumber mosaic virus" were probably strains of squash mosaic virus, but others were probably strains of watermelon mosaic virus. This conclusion is based on experience with virus-infected samples that infected squash, though not watermelon, in initial tests, but in further tests were found to infect watermelon systemically. Such samples might have been classified as squash mosaic virus if no recheck had been made.

Dickson et al (3), in a report on insect vectors of the virus complex surveyed by Middleton (15), said that *Diabrotica* beetles were extremely rare in the Imperial Valley, and that the most prevalent cantaloupe viruses were aphid-transmitted in a non-persistent manner. They also reported that virus-infected plants were very rare early in the season, and that only one seed-borne-mosaic-infected cantaloupe plant was found in the desert areas in 1949, even though many plants were observed.

Severin and Freitag (21), in 1948, described a strain of cucumber mosaic virus in the interior valleys of California that produced on sugar beet a systemic blister-mottle type of leaf distortion. They named the strain "Western Cucumber Mosaic Virus." In the past few years the present authors have collected many different samples of cucumber mosaic virus commonly occurring on a number of hosts in California. Different hosts have exhibited considerable variability in reaction to these collections, indicating a number of different strains. For example, some strains, producing systemic symptoms in beans and peas, are similar in that respect to the strains of cucumber mosaic virus described by Whipple and Walker (26). Other strains commonly occurring on celery in California are similar in host range and reaction to the cucumber mosaic virus strain described by Wellman (25) on celery in Florida. All of the collections of CMV that have been tested produced systemic symptoms on young sugar beet seedlings growing under warm temperatures. It is therefore our contention that the name "Western Cucumber Mosaic Virus" does not necessarily refer to any single strain of CMV, and that the name should not be used, because it suggests that a single strain of the virus occurs in the western part of the United States.

Recent reports by Rosberg (20) and by Shepherd and Struble (22) on the association of a pimples disease of watermelon with TRSV (tobacco ringspot virus) tell of isolating the virus from watermelon fruit with pimpled surfaces. However, neither report presented good evidence of inducing pimples by inoculations with TRSV.

Watermelons in California are commonly affected with a pimpling on the surface of fruits and stems. The authors have often attempted to recover TRSV by extracting juice from pimpled fruits and inoculating various host plants in the greenhouse. Inoculum has been taken from inside of the fruit to obtain the white tissue that Shepherd and Struble (22) said contained less inhibitor to TRSV than did green watermelon tissue. All tests were negative. In one test, graft-inoculations were made by inserting chips of tissue from pimpled fruits from the Imperial Valley into the stems of 10 healthy watermelon plants. Three developed watermelon mosaic; 7 did not. In other tests, juice from pimpled fruits from Davis, California, was inoculated to various hosts in the greenhouse, but no virus was recovered. It therefore appears to the authors that TRSV is not associated with the pimples disease of watermelon in California, which instead appears to be a physiological abnormality of unknown etiology.

Seed transmission of cucurbit mosaic viruses.—Cucumber mosaic virus has been reported to be transmitted to the seed of wild cucumber (*Echinocystis lobata*) (4, 5, 6). This has been confirmed by Lindberg, Hall, and Walker (12). Doolittle and Walker (6) failed to transmit the virus through the seed of cucumber, squash, muskmelon, or pumpkin, and concluded that seed transmission does not occur through these hosts, or is rare and of little significance.

TRSV is known to be seed-transmitted in some of its hosts (24), but not in watermelon seed (22), and only occasionally in cucumber seed (23).

Lindberg, Hall, and Walker (12) reported obtaining their "RMV" strain of WMV (watermelon mosaic virus) from an infected seedling, indicating that this strain may be seed-transmitted. However, the data reported herein indicate that strains of WMV are rarely, if ever, seed-transmitted.

Of the many reports on seed transmission of mosaic viruses by various cultivated cucurbits, probably most are concerned with strains of squash mosaic virus. The percentage of seed transmission has been variable. For example, Rader, Fitzpatrick, and Hildebrand (19) reported that transmission varied from 12 to 43% in seed from 3 single-plant selections of muskmelon, and from 27.9 to 93.5% in 2 other melon seed stocks. Mahoney (13) reported that seed transmission ranged from 1.0 to 17.0% in 5 different selections and averaged 27.1% in 5 other single-plant selections of melon. Keener (10) reported transmission of an unidentified virus by commercial seed samples of cantaloupe and Honey Dew melon. The percentage of transmission by Honey Dew seed ranged from 1.4 to 3.4% but the percentage transmission by cantaloupe seed was less than 1%.

There have been a number of reports from California on seed transmission of cucurbit viruses. In 1934 Kendrick (11) reported 0.23% seed transmission of an unidentified virus by commercial muskmelon seeds. In 1944 Middleton (14) reported that 0.96% of seed from virus-diseased squash plants transmitted squash mosaic virus, and added that Freitag had identified Kendrick's seed-transmitted virus as squash mosaic virus. In 1946 Middleton and Whitaker (17) reported 2.1–6.2% transmission of a virus by commercial seed stocks used to plant commercial acreage of Honey Dew melon in the Imperial Valley. They also reported that 1.4–4.7% of seed of PMR No. 5

cantaloupe transmitted virus, as did 18.5% of seed from another cantaloupe plant that they had harvested. Although they called the virus cantaloupe mosaic, one gets the impression that they thought it was the same virus that had been called squash mosaic virus by Middleton (14). It was inferred also that seed transmission was an important source of the virus affecting commercial cantaloupe fields in the Imperial Valley. However, the reported rarity of seed-borne-virus-infected cantaloupe plants in the Imperial Valley early in the season (3) indicates that the principal virus of cantaloupe in the area is rarely, if ever, seed transmitted.

Insect vectors of cucurbit mosaic viruses.—Some of the earlier reports (4, 6, 7) indicate that cucumber mosaic virus strains can be transmitted by *Diabrotica* beetles and aphids.

Freitag (7) reported that squash mosaic virus was readily transmitted by *Diabrotica* beetles, and infrequently by 5 species of aphids. Cucurbit ring mosaic virus was shown to be a strain of squash mosaic virus transmitted by beetles only (12). In 1952 Freitag (8) reported that squash mosaic virus and cucurbit ring mosaic were transmitted by beetles and not by aphids. In 1956 Freitag (9) reported that beetles transmitted squash mosaic virus in a persistent manner but did not transmit cucumber mosaic virus in several tests. The viruses that Freitag (8) called cantaloupe and western watermelon mosaic viruses and that Lindberg, Hall, and Walker (12) showed to be strains of watermelon mosaic, were transmitted by aphids and not by beetles. Freitag (8) described WMV as a nonpersistent aphid-borne virus transmitted during short feeding periods following a period of starvation. It seems quite unlikely, therefore, that the principal cucurbit mosaic virus in the Imperial Valley, which according to Dickson et al (3) is transmitted by aphids in a nonpersistent manner, could possibly be squash mosaic virus. In fact, the survey data presented by Middleton (15) as interpreted by the present authors indicate that the principal virus of cantaloupe and other cucurbits in the desert valleys of California is watermelon mosaic virus.

A SURVEY OF CUCURBIT MOSAIC VIRUSES IN WESTERN DESERT VALLEYS.—In 1956–58 samples of virus-infected cucurbit hosts were collected from the desert valleys of southern California and from the Yuma Valley of Arizona. A juice extract from each sample was inoculated to a differential host range in a greenhouse at Davis. The host range consisted of *Vigna sinensis* Savi (cowpea var. Black), *Nicotiana glutinosa* L., *Citrullis vulgaris* Schrad. (watermelon var. Striped Klondike), and *Cucurbita pepo* L. (pumpkin var. Small Sugar). From the reactions of these hosts the virus content of each collection was identified: as a strain of CMV from symptoms on all 4 hosts consisting of local lesion or occasionally systemic mottle on cowpea, severe stunting and necrotic spotting of young leaves of watermelon with subsequent recovery in new growth, systemic mottle and leaf distortion in *N. glutinosa*, and severe systemic yellow mottle and stunting of Small Sugar pumpkin; as a strain of WMV from no symptoms in cowpea and *N. glutinosa*, and systemic mottle in watermelon and Small Sugar pumpkin; as a strain of SMV from no reaction on *N. glutinosa* or cowpea, local lesions or no reaction on watermelon, and mottle and leaf distortion or, more commonly, a ringspot mottle of Small Sugar pumpkin; as a mixture of CMV and WMV from systemic mottle of new-growth of watermelon after other members of the host range had indicated CMV. The mixture could usually be proved by isolating WMV free of CMV in the growing tips taken from such watermelon plants.

The data from this survey, presented in Table 1,

TABLE 1.—*The occurrence and relative prevalence of several viruses of cultivated cucurbits in the desert valleys of Southern California and the Yuma Valley of Arizona*

Location and date of collection	Host	Number of collections	Frequency of recovery			
			Cucumber mosaic	Watermelon mosaic	Mixture of cucumber and watermelon mosaic	Squash mosaic
Imperial Valley 1956	Squash and Cantaloupe	97	12	84	0	1
Yuma Valley, Arizona 1956	Cantaloupe	8	0	8	0	0
Palo Verde Valley 1956	Honey Dew melon	13	0	13	0	0
Coachella Valley 1956	Squash	7	0	7	0	0
Imperial Valley 2/27/57	Squash	21	0	20	0	1
Imperial Valley 5/15/57	Watermelon	13	0	13	0	0
	Cantaloupe	28	1	13	13	1
Yuma Valley, Arizona 5/15/57	Cantaloupe	19	3	7	9	0
Palo Verde Valley 7/17/57	Cantaloupe	12	2	7	3	0
	Watermelon	12	0	12	0	0
Imperial Valley 2/23/58	Squash	20	0	20	0	0
Coachella Valley 2/23/58	Squash	3	0	3	0	0
Imperial Valley 3/31/58	Squash	38	2	36	0	0
Coachella Valley 5/11/58	Squash	8	0	8	0	0
Imperial Valley 5/11/58	Cantaloupe	46	1	43	2	0
Yuma Valley, Arizona 5/11/58	Cantaloupe	18	2	12	4	0
Totals		363	23	306	31	3

show conclusively that WMV was the most prevalent virus affecting cucurbit hosts in the desert valleys of California and the Yuma Valley of Arizona. It was present, alone or in combination with CMV, in 337 of the 363 samples; CMV was second-most prevalent, being present, alone or in combination with WMV, in 54 samples; SMV was present in only 3 samples (which could be accounted for by seed transmission, with little or no spread in the areas surveyed). These data, together with the report (3) that aphids were very abundant and cucumber beetles very rare in the desert valleys, tend to substantiate the reports (9, 12) that SMV strains are spread by beetles but not by aphids.

The relative prevalence of CMV and WMV, each virus either alone or in combination with the other, was fairly consistent during the 3 years of the survey —respectively: 9% and 89% of all samples in 1956, 29% and 93% in 1957, and 9% and 96% in 1958. The outstanding difference was the greater percentage of CMV in 1957.

DEVELOPMENTAL SEQUENCE OF MOSAIC IN CUCURBIT CROPS IN THE IMPERIAL VALLEY OF CALIFORNIA.—The incidence of mosaic infection on cantaloupe and other cucurbits in the Imperial Valley increases so rapidly that it can be likened to an explosion. The typical development in 1958 illustrates the usual process. Before it is described, note should be taken (Table 1) that the virus-infected specimens collected in February and March were all squash (*Cucurbita pepo*, varieties Zucchini, Bush Scallop, or Yellow Crookneck). The few cantaloupe and watermelon planted that early had not yet emerged or were still covered with hot caps. Therefore, the only virus-infected cucurbits that could be found were squash that had been planted under brush-protection for winter harvest. Some acreage, planted in August of the previous year, contained a large percentage of virus-infected plants. However, most of the squash had been seeded in December or January, and only a very small percentage of these were virus-infected. In fact, not a single virus-infected plant could be found in some of the plantings on February 23.

All the February collections yielded only WMV, except for a single collection of SMV, in February, 1957.

About 1 month later (March 31), the percentage of infected plants had increased greatly in the same squash plantings, with no planting virus-free. Collections at this time yielded 2 samples of CMV, and 36 of WMV.

Hot caps were removed shortly thereafter, and, according to Dr. G. W. Bohn, mosaic was first observed in cantaloupes on about April 15 (personal correspondence with Dr. Bohn). By May 11, when the area was revisited, most cantaloupe fields were heavily infected, making virus-free plants hard to find in many of them.

One might conclude that the winter squash plantings are the principal reservoir of the viruses that infect cantaloupe and watermelon planted later. However, the squash plantings are small, widely separated, and free of virus during early growth, indicating that there is some other source of WMV inoculum in the Valley.

The absence of wild cucurbitaceous hosts in the cultivated areas of the Valley suggested the possibility of non-cucurbit hosts of WMV in the native or cultivated flora. The results of a search for such hosts are reported in a later section.

TESTS FOR SEED-TRANSMISSION OF CURCURBIT VIRUSES. —*Tests of commercial seed or seed harvested from commercial fields by the authors.*—Commercial seed stocks of cantaloupe, Crenshaw melon, and Persian melon from two commercial seed companies were planted in sterilized soil in a greenhouse at Davis. About 500 seeds of each of 13 seed stocks were planted, from which 5720 seedlings emerged. Of these, 15 (about 0.3%) were virus infected. When saps extracted from the 15 virus-infected seedlings were each inoculated to a differential host range, none reacted on cowpea or *N. glutinosa*, but most gave local lesions on watermelon and a systemic ringspot mottle on squash, typical reactions of these hosts to the cucurbit ringspot strains of SMV (12).

In another test 10 different seed samples of Crenshaw, Zucchini squash, cantaloupe, and pumpkin were planted in a greenhouse bench as previously described. Of 2612 seedlings that emerged, 8 (about 0.3%) were virus-infected. When inoculated to the differential host range, all 8 samples were identified as the cucurbit ringspot strain of SMV.

In the Yuma Valley of Arizona in 1957, 1 fruit from each of 5 virus-infected cantaloupe plants was collected, and samples of leaf tissue were taken from the same 5 plants. The viruses in the leaf samples were identified as follows: 4 plants were infected with both CMV and WMV; 1 plant was infected with WMV alone. Seed from the 5 fruit, bulked and planted in a greenhouse bench of sterilized soil, produced 1250 seedlings, none of which were virus infected.

In 1956, 496 seed samples were received from Dr. T. W. Whitaker, each containing 24 cantaloupe seeds harvested from virus-infected plants at the Southwestern Irrigation Field Station, Brawley, in the Imperial Valley. About 11,000 seedlings were produced therefrom, and carefully observed for 1 month after emergence. None were virus infected. Since surveys in the Imperial Valley in 1956, 1957, and 1958 have shown that strains of WMV and CMV are prevalent there, it seems likely that the plants from which the seed was obtained were infected with these viruses. It appears, therefore, that neither of these viruses is commonly transmitted in cantaloupe seed.

Transmission by seed produced on inoculated plants. —The above data indicate that SMV is commonly transmitted in commercial seed stocks grown in California. They also indicate that CMV and WMV are rarely, if ever, transmitted in cantaloupe and other cucurbit seed. To obtain more precise data on virus

TABLE 2.—*Amount of virus transmission through seeds of several cucurbit hosts mechanically inoculated with squash or watermelon mosaic virus*

Source of seed	Squash mosaic virus			Watermelon mosaic virus		
	No. seedlings	No. virus infected	% seed trans.	No. seedlings	No. virus infected	% seed trans.
Cucurbita maxima Duch. (Buttercup squash)	452	6	1.3	518	0	0
Cucurbita maxima Duch. (Hubbard squash)	518	1	0.2	405	0	0
Cucurbita maxima Duch. (pink banana squash)	459	7	1.5	408	0	0
Cucurbita mixta Pang. (variety unknown)	219	0	0.0	484	0	0
Cucurbita mixta Pang. (Tennessee sweetpotato squash)	295	1	0.3	431	0	0
Cucurbita moschata Duch. (butternut squash)	275	0	0.0	503	0	0
Cucurbita pepo L. (zucchini squash)	78	4	5.1	194	0	0
Cucurbita pepo L. (Early summer golden crookneck squash)	529	0	0.0	588	0	0
Cucumis melo L. (honeydew melon)	356	24	6.6	(No fruit set)		
Cucumis melo L. (Rocky Ford cantaloupe)	431	89	20.7	(No fruit set)		
Citrullus vulgaris Schrad. (Klondike watermelon)	(Not virus infected)			(No fruit set)		
Cucurbita andreana Nound.	10	0	0.0	65	0	0
Cucurbita sororia Bailey	159	0	0.0	176	0	0
Totals	3791	132	3.5	3772	0	0

transmission in seed from plants known to be infected with WMV or SMV at an early stage, the following work was done: The cucurbitaceous hosts listed in Table 2 were planted in a greenhouse, and the first true leaves were inoculated mechanically with either SMV or WMV. After symptoms had developed, the seedlings were transplanted to a field near Davis, California for seed production. Shortly before the fruits were harvested, samples of tissue from each of the mother plants were inoculated to a differential host range in the greenhouse to confirm infection with either SMV or WMV. Each was infected with the virus inoculated to it, except that the watermelon plants were not infected with SMV. Seed samples from each infected plant were sown in the greenhouse to test for seed transmission. The results are in Table 2. WMV was not transmitted by seed of any of the species and varieties. SMV was transmitted through several but not all, with extremely variable transmission, ranging from 20.7% (Rocky Ford cantaloupe) to none (5 hosts).

HOST RANGE OF STRAINS OF WMV.—Anderson (2) listed a number of cucurbits that are susceptible to strains of WMV, but hosts outside the family *Cucurbitaceae* have not been reported heretofore.

The failure of the virus to transmit through cucurbit seed, the absence of wild cucurbits near cultivated areas of the Imperial Valley, and the scarcity of virus-infected squash and cantaloupe plants during the first month or so of the growing season suggested that hosts other than cucurbits are susceptible to the virus.

To test this hypothesis, juice from Small Sugar pumpkin plants recently infected with collection 9 of WMV (Table 3) was mechanically inoculated to the following species, representing 7 families: UMBELLIFERAE—*Daucus carota* L., carrot, var. Long Imperator; *Apium graveolens* L., celery vars. Utah 10B and Golden Self-Blanching; *Coriandrum sativum* L., coriander; *Anthriscus cerefolium* Hoffm., salad chervil. LEGUMINOSAE—*Melilotus indica* All., sour clover; *Melilotus officinalis* Lam., yellow sweet clover; *Medicago sativa* L., alfalfa var. African; *Medicago hispidia* Gaerton, burr clover; *Sesbania macrocarpa* Muhl.; *Vigna sinensis* Savi, cowpea var. Black; *Lathyrus odoratus* L., sweet pea; *Vicia faba* L., broadbean; *Pisum sativum* L., garden pea var. Wyoming Wonder; *Lupinus hirsutus* L., Blue lupine; *Phaseolus vulgaris* L., bean var. Bountiful; *Phaseolus limensis* Maef., Lima bean var. Henderson Bush; *Trifolium hybridum* L., alsike clover; *Trifolium incarnatum* L., crimson clover; *Trifolium pratense* L., red clover; *Trigonella foenum-graecum* L., fenugreek; *Cyamopsis psoraloides* DC., guar. MALVACEAE—*Gossypium hirsutum* L., cotton var. Acala 442; *Hibiscus sabdariffa* L., rozelle; *Hibiscus esculentus* L., okra var. White Velvet; *Malva parviflora* L., cheeseweed. SOLANACEAE—*Capsicum annuum* L., pepper var. California Wonder; *Petunia hybrida* Vilm., petunia; *Solanum tuberosum* L., potato var. USDA Seedling 41956; *Nicotiana glutinosa* L., *Nicotiana clevelandii* Gray. COMPOSITAE—*Coreopsis tinctoria* Nutt. tickseed and *Emelia* sp. GRAMINEAE—*Hordeum vulgare* L., barley var. Atlas 46; *Zea mays* L., sweet corn var.

Golden Cross Bantam. CHENOPODIACEAE—*Beta vulgaris* L., sugar beet var. U. S. 400.

Thirteen days after inoculation the leaves of garden pea were definitely mottled; the leaves of crimson clover, alsike clover, red clover, and okra appeared slightly mottled, but too indistinctly to be recorded as definite.

Three weeks after inoculation of the test plants, Small Sugar pumpkin seedlings were mechanically inoculated with sap extracted from each of the varieties and species in the test to determine if WMV could be recovered. The virus was recovered from fenugreek, sweet pea, crimson clover, pea, lupine, guar, coriander, and chervil.

To determine whether the host ranges of other collections of WMV are similar to that of collection 9, 12 different collections of WMV (Table 3) were inoculated to the various host plants listed in Table 4. Inoculum source of each of the 12 viruses was Small Sugar pumpkin plants recently infected. After inoculation the various test plants were observed for symptom expression, and sap extracts from any that appeared to be virus infected and from some that were symptomless were inoculated to Small Sugar pumpkin plants to check for recovery of the viruses. Results are presented in Table 4. All of the collections except number 5 were recovered from at least some of the varieties and species outside of the *Cucurbitaceae*. There was considerable difference, however, in the host range exhibited by the viruses. For example, collection 8 was recovered from 14 of the 17 species, but collection 5 was recovered from only the 3 cucurbitaceous species. Anderson's and Freitag's strains of WMV were somewhat more restricted in host range than some of the California collections, but were recovered from 3 species of the *Leguminosae*.

Sweet pea, garden pea, and guar were infected by more collections than any other members of the Leguminosae, and in the Malvaceae, rozelle and malva were susceptible to more collections than were okra or cotton. Cotton showed a leaf mottling suggesting virus infection, but is not considered a host of WMV; the virus was not recovered from cotton by either mechanical or aphid inoculations to Small Sugar pumpkin seedlings.

Repeated tests probably would show that more of the plant species in the tests are susceptible to more of the virus collections than these data indicate, and the host range of strains of WMV undoubtedly includes species not tested.

These data do show, however, that WMV is composed of a number of strains, readily infecting members of the Cucurbitaceae but differing in their ability to infect species in other plant families.

WMV symptoms on several hosts.—Eight of the 12 WMV collections listed in Table 4 were recovered from sweet pea, but none produced any observable symptoms on this host. Crimson clover was faintly mottled by several of the collections, but virus was not recovered from all that showed symptoms. Collection 3, originally isolated from sour clover, produced definite mottle in this host; 1 and 2 produced only mild mottle; 6 produced no symptoms. None of the collections produced symptoms on yellow sweet clover, burr clover, or sesbania, and only collection 8 was recovered from these hosts. In all instances in which a virus collection was recovered from garden pea, definite symptoms were evident: 1) distinct mottle with no leaf necrosis, or 2) severe mottle, leaf necrosis, and severe stunting. Symptoms produced in broadbean and vetch were very indistinct, ranging from no obvious symptoms to very faint mottle. Symptoms in lupine ranged from a very mild to a distinct mottle. Some collections produced definite mottle in guar, but most induced only a very mild and indistinct mottle. None of the collections produced definite symptoms in rozelle, but 7 of the 12 collections were reisolated from this host. Six of the collections produced mottle and leaf clearing in okra, but only 2 were recovered. *Malva parviflora* showed yellow mot-

TABLE 3.—*Source of watermelon mosaic virus collection used in host range tests*

Virus collection	Source of collection
Anderson's WMV	Kindly furnished to authors by Dr. J. C. Walker. It is a subculture of WMV received by Lindberg et al (12) from C. W. Anderson, of Florida.
Freitag's WMV	Kindly furnished by Dr. J. H. Freitag. Probably a subculture of WMV (12).
#1	Isolated in 1957 from alfalfa collected at Southwestern Irrigation Field Station in Imperial Valley near Brawley, California.
#2	Isolated in 1957 from *Malva parviflora* collected near Brawley, California.
#3	Isolated in 1957 from *Melilotus indica* collected near Brawley, California.
#4	Isolated in 1957 from mottled and misshapen watermelon fruit collected near El Centro (Imperial Valley), California.
#5, 6, 7	Isolated in 1957 from squash collected near Niland (Imperial Valley), California.
#8	Isolated in 1957 from squash collected near Brawley, California.
#9	Isolated from squash collected near San Bernardino (San Bernardino Co.), California.
#10	Isolated in 1957 from watermelon collected near Woodland (Yolo County), California.

tling and yellow spotting of the leaves, and the virus was not recovered from any symptomless plants. All of the strains produced mottle and leaf distortion on watermelon, and in addition some produced a necrotic flecking of the leaves of some plants. Inoculum taken from plants showing leaf necrosis and reinoculated to watermelon usually produced only the typical mottle and leaf-cupping symptoms, indicating that the necrotic reaction in watermelon may be due to a difference in genetic makeup of individual plants rather than to differences in strains of the virus. All of the strains produced only mottle and leaf distortion symptoms on citron, with no necrosis. All of the collections produced similar symptoms on Small Sugar pumpkin, consisting of a distinct mottle but only slight stunting, and eventually raised, green, blister-like areas developed on the leaves.

Recovery of WMV and CMV strains from non-cucurbits in nature.—During 1956 CMV was recovered (by mechanical inoculation) from 2 samples of *Solanum elaegnifolium* Cav. collected in the Imperial Valley near Holtville, California. No other recoveries were made that year in 22 other attempts to recover cucurbit-infecting viruses from samples of non-cucurbitaceous hosts suspected of being virus-infected. The other host plants collected from several locations in the Imperial Valley and number of samples of each tested were: 1 *Baccharis glutinosa* Pers., 4 *Erigeron candensis* L., 8 *Helianthus annuus* L., 1 *Brassica campestris* L., 1 *Melilotus alba* Desr., 2 *Lactuca serriola* L., 1 *Aster spinosus* Benth., 3 *Chenopodium album* L., and 1 *Malva parviflora* L.

Since alfalfa (*Medicago sativa* L.), sour clover (*Melilotus indica* All.), and cheeseweed (*Malva par-*

TABLE 4.—*Recovery of watermelon mosaic virus strains from mechanically inoculated plants in host-range tests*[a]

Hosts	Virus collection												Total recovered	Total attempts
	Anderson's WMV	Freitag's WMV	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10		
Leguminosae														
Sweet pea	+	+	+	+	+	–	–	+	–	+	+	–	8	12
Crimson clover	+	–	+		–	–	–	–	–	+	+	–	4	11
Sour clover	–	–	+	+	+	–	–	+	–		–	–	4	11
Yellow sweet clover	–	–	–	–	–	–	–	–	–	+	–	–	1	12
Bur clover	–	–	–	–	–	–	–	–	–	+	–	–	1	12
Garden pea	+	–	+	+	+	–	–	+	+	+	+	–	8	12
Vicia faba	–	–						+		+			2	4
Vetch	–	+	+				–						2	4
Sesbania	–	–	–	–	–	–	–	–	–	+	–	–	1	12
Blue lupine	–	+		+	+	–	–	–	–	+	+	+	6	11
Guar	–	–	+	+	+	–	–	+	–	+	+	+	7	12
Alfalfa	–	–	–	–	–	–	–	–	–	–	+	–	1	12
Malvaceae														
Cotton	–	–	–	–	–	–	–	–	–	–	–	–	0	12
Rozelle	–	–	+	+	+	+	–	+	–	+	+	–	7	12
Okra	–	–	+	+	–	–	–	–	–	–	–	–	2	12
Malva	–	–	+	+	+	–	–	+	–	+	+	–	6	12
Cucurbitaceae														
Watermelon	+	+	+	+	+	+	+	+	+	+	+	+	12	12
Citron	+	+	+	+	+	+	+	+	+	+	+	+	12	12
Small sugar pumpkin	+	+	+	+	+	+	+	+	+	+	+	+	12	12
Total recovery attempts of each virus	19	19	16	16	17	17	18	18	17	17	17	17		
Total successful recoveries of each virus	6	6	12	11	10	4	3	11	4	14	10	5		

[a] + indicates virus was recovered; blank spaces indicate no attempt to recover virus was made.

viflora L.) had proved susceptible to several collections of WMV when inoculated mechanically in the greenhouse (Table 4), a number of samples of these hosts were collected in the Imperial Valley during the 1957 season to determine whether the virus could be recovered from them in nature. Mechanical inoculations made to a differential host range with sap extracts from these samples gave the following results: of 13 samples of sour clover, 1 yielded WMV, 9 yielded alfalfa mosaic virus, and 3 yielded no virus; of 5 samples of *Malva parviflora*, 1 yielded WMV and 4 yielded no virus; of 10 samples of alfalfa, 1 yielded WMV, 2 yielded alfalfa mosaic virus, and 7 yielded no virus; 7 samples of sugar beet, which was not susceptible to WMV in greenhouse inoculations, yielded no virus.

During the 1958 season 25 samples of alfalfa with mottled or distorted leaves were collected near cantaloupe fields in the Imperial and Palo Verde valleys. A portion of each of the samples was used for mechanical inoculation of a differential host range; the other portion was exposed to previously starved green peach aphids (*Myzus persicae*), which were transferred after a short feeding period to healthy Small Sugar pumpkin plants. WMV was recovered from 2 of the 25 samples by both mechanical and aphid transfer. Attempts failed to recover virus from 23 samples of *Melilotus indica*, 12 of *Malva parviflora*, 2 of *Chenopodium album*, and 1 of *Sonchus oleraceous*.

Since WMV is rarely, if ever, transmitted through the seed of its cucurbitaceous hosts, recovery of the virus from the relatively few samples tested of alfalfa, *Melilotus*, and *Malva* indicates that these, and possibly other, non-cucurbitaceous hosts may be sources of inoculum for melon crops in nature.

Middleton (15) stated that he had secured SMV (called "watermelon mosaic virus" in this paper) from *Cucurbita palmata* Wats. and *Cucurbita foetidissima* H. B. K. However, since neither of these species occurs commonly in the cultivated areas of the Imperial or Palo Verde valleys, it seems unlikely that they are important as inoculum sources of the virus.

PHYSICAL PROPERTIES OF STRAINS OF WMV.—The physical properties, inactivation *in vitro* at 21°C, inactivation by heating 10 minutes at 50°, 60°, or 70°C, and inactivation by dilutions of 10^{-3}, 5×10^{-3}, or 10^{-4}, were determined for 26 WMV collections from California, 2 from Mexico, and for the Anderson and Freitag strains of WMV. All 30 of the collections had given reactions typical of strains of WMV on the differential host range, and had been transmitted in greenhouse tests by short feeding periods of green peach aphids (*Myzus persicae*). Recently infected *Cucurbita pepo* L. (var. Small Sugar) was the source plant for each virus, and virus activity after the various treatments was determined by inoculations to young Small Sugar pumpkin seedlings.

All 30 virus collections were active at a dilution of 10^{-3}, 14 were active at 5×10^{-3}, and 19 were active at 10^{-4}.

Seventeen of the viruses were active after heating at 50°C, but all 30 were inactivated by heating at 60° or 70°C.

All 30 controls (0 time) in tests of inactivation *in vitro* at 21°C were active; 14 were active after 10 days, 4 were active after 20 days, but none were active after 30 days.

Freitag's WMV strain was active at the 10^{-3} dilution, but not at the higher dilutions, whereas Anderson's strain was active at all 3 dilutions. Neither strain survived any of the heat treatments. Anderson's strain did not survive 10 days of storage *in vitro* at 21°C, but Freitag's strain was active after 20 days storage.

These data show considerable variability in the physical properties of the different collections. They are not in complete agreement with the physical properties of strains of WMV reported by Lindberg, Hall, and Walker (12), but fall within the range of variability of a group of strains of a virus.

PHYSICAL PROPERTIES AND INSECT TRANSMISSION OF STRAINS OF SMV.—Ten cantaloupe seedlings infected with seed-borne virus were obtained from a greenhouse soil bench planted with commercial seed samples of several varieties of cantaloupe. All 10 virus samples, when inoculated to the differential host range, gave reactions typical for the cucurbit ring mosaic strain of SMV (12). In a single attempt, 3 of the 10 virus samples were transmitted to healthy Small Sugar pumpkin seedlings by *Diabrotica undecimpunctata* Mann. beetles that had been given a 5-hour acquisition feeding period on the virus sources and a 1-hour transmission feeding period. None of the 10 virus samples were transmitted by short feeding periods of green peach aphids that had been starved prior to a short acquisition feeding period.

Physical properties of the 10 virus samples were as follows: 7 of 10 samples were still active after heating 10 minutes at 60°C, but all were inactivated by 10 minutes at 70° or 80°C; all 10 samples were active after aging *in vitro* at 21°C for 20 days, and 5 were still active after 40 days of such aging.

OCCURRENCE OF WMV IN MEXICO.—Deformed fruit and poor fruit set of watermelons and squash in Mexico have been the occasion for several inquiries from watermelon growers and commercial seedsmen. The symptom descriptions received have suggested WMV as a likely cause of the trouble. A permit was obtained from quarantine officials to receive samples of tissue to test for virus content. Samples from Los Mochis, in Sinaloa, and Guaymas, in Sonora, were indexed on the differential host range with the following results: of 19 samples of squash from Los Mochis, 16 had WMV, 1 had SMV, 1 had CMV, and 1 indexed negative; 6 samples of squash from Guaymas yielded nothing, but WMV was recovered from 1 sample of *Melilotus indica* and from 1 sample of watermelon.

Symptoms on Small Sugar pumpkin produced by the WMV isolates from Mexico were very similar to

TABLE 5.—*The occurrence and relative prevalence of several viruses of cultivated cucurbits in central and northern California*

Collection				Frequency of recovery of		
Location	Date	Hosts	Number of collections	Cucumber mosaic virus	Squash mosaic virus	Watermelon mosaic virus
West side San Joaquin Valley, near Patterson	8/2/56	Cantaloupe	7	0	7	0
Sutter County, near Yuba City	7/10/56	Honey Dew	8	7	1	0
Yolo County, near Davis	7/15/56	Squash	2	0	2	0
		Cucumber	1	1	0	0
		Cantaloupe	4	1	3	0
San Benito County, near Gilroy	8/1/57	Cucumber	12	12	0	0
Yolo County, near Woodland	7/25/57	Watermelon	4	1	0	3
		Cucumber	3	2	1	0
		Honey Dew	16	16	1[a]	3[a]
Totals			57	40	15	6

[a] Mixed with cucumber mosaic virus.

those produced by California isolates of WMV; symptoms on watermelon were sometimes somewhat more severe. Otherwise there was little difference between California and Mexican isolates. All of the Mexican isolates of WMV were transmitted by green peach aphids in greenhouse tests, and determinations of the physical properties of 2 of them showed close agreement with the physical properties of 28 California isolates.

The occurrence of WMV in 2 widely separated valleys in Mexico indicates that the virus may be widely distributed in the irrigated desert valleys of the West Coast of Mexico.

SURVEY OF CUCURBIT MOSAIC VIRUSES IN CENTRAL AND NORTHERN CALIFORNIA.—Limited surveys were made in 1956–57 to determine the prevalence and identity of cucurbit mosaic viruses in cucurbit crops in central and northern California. Samples were indexed for virus content on the differential host range used in surveying the desert areas. The data (Table 5) are too few to give a true indication of relative prevalence in the several locations where samples were taken, but are so strikingly different from those obtained in the desert areas there can be little doubt that the differences are significant. These data indicate that CMV is the most prevalent virus of cucurbits in northern California, with SMV more prevalent in the Patterson area. WMV occurred occasionally in watermelon and Honey Dew near Woodland, but CMV was much more prevalent, especially in Honey Dew melon. The only instance in which CMV or any virus but WMV was obtained from watermelon (Table 2) was from a small planting of watermelon in the corner of a large field of Honey Dew melons heavily infected with CMV.

CROSS-PROTECTION TESTS.—Several virus collections identified as strains of CMV on the differential host range were subjected to cross-protection tests to check the accuracy of identification. *Vigna sinensis* (cowpea variety Black) were systemically infected either with strain 14 of CMV (26) or with a strain (isolated from pepper collected in 1957 near Tulare, California) that produces systemic infection of bean, garden pea, and cowpea as does strain 14. After 20 days the test plants were reinoculated with 11 different virus collections that produced local lesions on cowpea and typical CMV symptoms on other hosts. Inoculations were made to healthy cowpea plants of the same age at the time the challenge inoculations were made. Abundant local lesions were produced on both primary and trifoliate leaves of the healthy plants, but very few, or usually none, on plants previously infected with strain 14 or the pepper isolate of CMV.

Strain 14 also prevented local-lesion production by 2 CMV strains from Wisconsin and by the P6 strain of CMV that has been used in cross-protection tests by other workers with zinnia as the test host.[3]

The natural occurrence of CMV and WMV together in cantaloupe plants raised the question as to possible strain relationships of the 2 viruses. Cross-protection tests were made by challenge inoculations with CMV on watermelon plants systemically infected with strains of WMV. Three collections of WMV were tested against challenge inoculation with 3 collections of CMV. Abundant local lesions were produced on watermelon, whether or not previously infected with WMV, indicating no close relationship.

Strain 14 of CMV failed to prevent local-lesion production on cowpea by a strain of alfalfa mosaic virus isolated from Ladino clover collected near Davis in 1956, indicating no close relationship although host range, host reactions, and physical properties had suggested the possibility.

DISCUSSION.—The survey data from California, showing WMV more prevalent in some areas and CMV more prevalent in others, are similar to data presented by Anderson (1) indicating greater prevalence for

[3] These 4 virus strains were obtained from Dr. Glenn S. Pound of the University of Wisconsin.

WMV in central Florida, and for CMV in the Orange-Seminole-Volusia area. He suggested several possible explanations for the situation in Florida, but they fail to explain the distribution and relative prevalence in California. For example, in Florida, WMV-infected *Melothria* spp. were especially common where WMV was most prevalent, indicating that it is an important source of the virus. In California, however, wild cucurbits occur very infrequently in or very close to cultivated desert areas where WMV is the predominant virus on cultivated cucurbits. Anderson (1) indicated also that WMV seemed to spread more rapidly than CMV in squash plantings, and that older squash were more resistant to CMV than to WMV. If so, this might explain why WMV is more prevalent in the Imperial Valley, where squash is grown as a winter crop and becomes heavily infected with WMV before cantaloupe and other cucurbits are exposed to infection. However, since WMV is also the predominant virus of Honey Dew melon and cantaloupe in the Palo Verde Valley, where practically no squash is grown, there probably is some other reason for the predominance of WMV in both areas.

The scarcity of WMV-infected cucurbits early in the growing season, and evidence that the virus occurs in non-cucurbit hosts in nature, suggest that the primary inoculum for WMV may come from non-cucurbit hosts. Since it is likely that most of the primary inoculum of CMV also comes from non-cucurbit hosts, it seems probable that the relative prevalence of both viruses may depend largely on the composition of the native and cultivated flora in which the viruses persist when cucurbits are not being grown. WMV might predominate where the flora is more favorable for its persistence and widespread distribution; CMV might be the predominant virus where it is favored. The fact that WMV was isolated more frequently than CMV from samples of non-cucurbits collected in the Imperial Valley suggests that the flora of the valley favors its perpetuation and tends to explain why it is more prevalent there in cucurbits. It suggests also that many of the hosts of CMV demonstrated by artificial inoculation in greenhouse tests do not serve as perpetuating hosts in nature. If they did, CMV should be more prevalent than WMV in practically all areas.

Melilotus indica, a very common weed in the Imperial Valley, seems to be an example of a species that is a more suitable natural host for WMV than CMV, although it is susceptible to both viruses in greenhouse tests. The most prevalent virus affecting this host in the Imperial Valley was alfalfa mosaic, but a few samples yielded WMV. However, none that were collected and analyzed by inoculation on a differential host range were found to be infected with CMV. Since none of the virus-infected cucurbit samples from the Imperial Valley were infected with alfalfa mosaic virus, indications are that cucurbits are refractory to infection with strains of this virus. Otherwise it might have been more prevalent than either CMV or WMV in cucurbits.

The occurrence of WMV in 2 widely separated valleys in Mexico south of the Imperial Valley of California suggests that the virus may be indigenous to the desert areas of California and Mexico.

Since WMV is rarely, if ever, seed-transmitted, and can persist in several non-cucurbit hosts while cucurbits are not being grown, the only control measure with much likelihood of success is the development of resistant varieties. Whitaker and Bohn (27) reported that several accessions of *Cucumis melo* var. *conomon* (Thumb.) Makino (Oriental pickling melon) were tolerant or resistant to mosaic when exposed to natural infection in the Imperial Valley. The progeny from some of these P.I. accessions (kindly furnished by Whitaker and Bohn) have shown some tolerance or klendusity to WMV in greenhouse inoculation tests, but none were tolerant to CMV. Much more work needs to be done to find a higher level of tolerance to both viruses and to incorporate it into commercially acceptable types of *Cucumis melo*.

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FACTORS AFFECTING DEVELOPMENT AND MORPHOLOGY OF REPRODUCTIVE STRUCTURES OF THE SOYBEAN ROOT AND STEM ROT PHYTOPHTHORA[1]

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SUMMARY

Some factors affecting the morphology of the *Phytophthora* species inciting root and stem rot of soybeans were investigated. Type of medium and source of water affected formation of sporangia. Sporangial papillation was variable and affected by many factors. Type of medium, source of the fungus, and age of culture affected type of sporangia germination; type of medium and age of culture influenced the size of sporangia. Type of medium, especially the percentage of agar, influenced the frequency of oogonia and diameter of oospores. Intercalary sporangia and resting sporangia were observed.

In 1951, a new root and stem rot of soybean was observed in one county in Northwestern Ohio; in 1955, Suhovecky (7) reported that a species of *Phytophthora* was the incitant. The soybean *Phytophthora* has recently been variously described (1, 2, 5, 7) and variously identified (1, 2).

Herr (1) identified an Ohio isolate as *Phytophthora cactorum* (Leb. & Cohn) Schroet., and reported the antheridia to be paragynous. Growth rate and diameter of oospores were different from those of *P. cactorum* as described in the literature, but were considered minor since the Ohio isolate resembled descriptions of *P. cactorum* very closely in many other aspects. Tucker (8) said: "*P. cactorum* may be identified by its profuse production of oogonia with paragynous antheridia, the absence of typical chlamydospores, the small and often inconspicuously papillate sporangia, and by its growth on corn meal agar at 27.5°C. The only other species with predominantly paragynous antheridia is *P. syringe*, which does not grow at 27.5°C."

Kaufmann and Gerdemann (2) reported a root and stem rot of soybean incited by *Phytophthora sojae* n. sp. Kauf. in Illinois, and said that isolates of soybean *Phytophthora* from Ohio, North Carolina, and Illinois were indistinguishable; the antheridia were reported to be paragynous, and the sporangia as ovoid or ellipsoid, with broad indistinct papillae. These characters were considered constant. They reviewed descriptions of all species considered synonymous with *P. cactorum* as well as other species having paragynous antheridia and concluded that the soybean *Phytophthora* was distinct.

Leonian (3) believed that there were not more than three good species of *Phytophthora*, i.e., *P. infestans*, *P. cactorum*, and *P. palmivora*, although 22 species were listed in his key. He said: "The genus *Phytophthora* does not readily lend itself to a natural scheme of classification. Too few morphological distinctions and the too ready tendency of such distinctive lines to overlap have necessitated a more or less arbitrary method of classification."

Several keys are available for identifying *Phytophthora* spp. (3, 4, 8, 11). Waterhouse (10) listed diagnoses (or descriptions) and figures of *Phytophthora* spp. from the original papers. Waterhouse and

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Blackwell (11) said: "There has been no comprehensive account of the species of *Phytophthora* since Tucker's monograph (1931), which remains the standard work. . . ."

Measurements of the various morphological forms of the soybean *Phytophthora* are discussed by Herr (1), Kaufmann and Gerdemann (2), Skotland (5), and Suhovecky (7).

Waterhouse (9) reported on the factors affecting the production of sporangia in the genus *Phytophthora*. Herr (1) had difficulty in obtaining sporangia of the incitant of root and stem rot of soybean. Kaufmann and Gerdemann (2) reported that "Sporangia of the soybean *Phytophthora* formed only occasionally in culture tubes on hemp seed in water, but when these cultures were poured into Petri plates, often numerous sporangia appeared in about one week."

To resolve conflicting reports on the morphology of the species involved and to establish valid taxonomic criteria for identification, more detailed observations were made on the morphology of the fungus, and especially on some of the factors affecting the development and morphology of the reproductive structures.

MATERIALS AND METHODS.—The source of the pathogen used in the experiments, unless otherwise specified, was a subculture of Suhovecky's (7) isolate maintained on PDA (potato-dextrose agar) slants containing 5 ml of medium. Small pieces of such cultures were used as transfer material to various media.

Other sources of transfer material were pieces of cultures taken from the edges of Lima-bean, corn, or soybean agar plates. The media consisted of 50 Fordhook bush Lima-bean seeds or M-15 corn kernels or 5 grams Lincoln soybean seed in 1 liter of distilled water with 20 g agar added. After autoclaving, the liquid was carefully decanted into Petri plates.

Unless otherwise specified, all media were autoclaved 20 minutes at 15 lb. pressure. Immediately after being autoclaved, about 20 ml were poured into each Petri plate.

Water was distilled in an "improved 'precision' water still" and stored in glass carboys.

EXPERIMENTAL RESULTS.—During the research, oogonia were observed with paragynous antheridia (Fig. 1-A). Antheridia occasionally appeared to be amphigynous, but more detailed examination revealed that they were paragynous. Sporangia formed rarely or not at all on media containing 1.5% or more of agar. Proliferation was not conclusively demonstrated. Some sporangia appeared to proliferate, but the sporangiophores were found on more detailed examination to resume growth outside of and below the emptied sporangia (Fig. 1-M). Conspicuously papillate (Fig. 1-E–H) and inconspicuously papillate (Fig. 1-B–D) sporangia were both observed. The papillae were generally broad, but narrowly papillate sporangia could be found occasionally. The sporangia germinated by germ tubes and zoospores (Fig. 1-L) and abortive sporangia were observed in which the undifferentiated protoplasm (Fig. 1-K) was extruded. Intercalary sporangia (Fig. 1-J) similar to the sphaeroconidia described by Blackwell and Waterhouse (2), and resting sporangia (Fig. 1-I) similar to the resting conidia described by these authors, were also observed.

The agar and Lima-bean extract content of the medium affected oogonial and sporangial development. Media were prepared of varying amounts of Fordhook bush Lima beans combined with varying amounts of agar. High agar was 17 g per liter; low agar was 1.7 g per liter. High Lima-bean was 62.5 g per liter; and low Lima-bean was 6.25 g per liter. Pieces of cultures on PDA were used as transfer material. The approximate number of oogonia and sporangia that developed on the various media at room temperature in 7 days were observed. Oogonia developed in abundance on media of high concentration of Lima-bean extract regardless of agar percentage. Sporangia developed in abundance in low Lima-bean extract only when the agar content was low or the agar was omitted. Media more favorable for oogonial development were less favorable for sporangial development.

Data are presented in Table 1 on the range and mean diameter of oospores on various Lima-bean agar media. Range and mean diameters were both affected by the type of medium, and mean diameter was influenced more by agar than by Lima-bean extract.

Sporangial size was affected by both the medium and the age of the culture. Newly purchased Difco Lima-bean agar, control number 433340; 5-year-old Difco Lima-bean agar, control number 384288; and 24-year-old Difco Lima-bean agar, control number 21560, were used at the rate of 2.3 g per liter. In Table 2 are data on sporangial size and papillation. Media influenced the range and mean size of the sporangia, but many factors must influence sporangial papillation. The mean size of sporangia decreased with the age of the culture.

Germination of sporangia was also affected by the various batches of Difco Lima-bean agars. About 200 sporangia developed per plate on all of these media after 3½ days at room temperature. Sporangia developed on the newly purchased and the 5-year-old Difco Lima-bean agars consistently germinated with the formation of zoospores, but sporangia developed on the 24-year-old Difco Lima-bean agar always aborted; the protoplasm did not differentiate, but was extruded as a granular mass (Fig. 1-K).

TABLE 1.—*The effect of Lima-bean agars of varying composition on the range and mean diameters of oospores of the soybean Phytophthora*

Type of Lima-bean agar[a]	Range (μ)[b]	Mean (μ)[b]
High agar, high Lima-bean extract	18.7–46.2	31.7
High agar, low Lima-bean extract	12.5–42.5	29.3
Low agar, high Lima-bean extract	15.0–40.0	27.8
Low agar, low Lima-bean extract	7.5–36.2	25.2

[a] High agar was 17 g per liter; low agar was 1.7 g per liter. High Lima-bean extract was 62.5 g per liter; low Lima-bean extract was 6.25 g per liter.
[b] Based on 200 mature oospores.

Since various batches of the same medium variously affected sporangial germination, the effect of the source of the pathogen and the type of medium was investigated by transferring the pathogen from various sources to Petri plates containing various substrates. The source of the pathogen was the fungus growing on PDA, corn, Lima-bean, and soybean agar plates and diseased tissues. The substrates consisted of tap water, distilled water, 2.3 g per liter of Difco Lima-bean agar prepared with tap water, and the latter prepared with distilled water. The cultures were maintained for 3½ days at room temperature. The source of the pathogen used for transfer material affected both the development of sporangia and the type of germination. The immediate medium used also affected sporangial development and type of

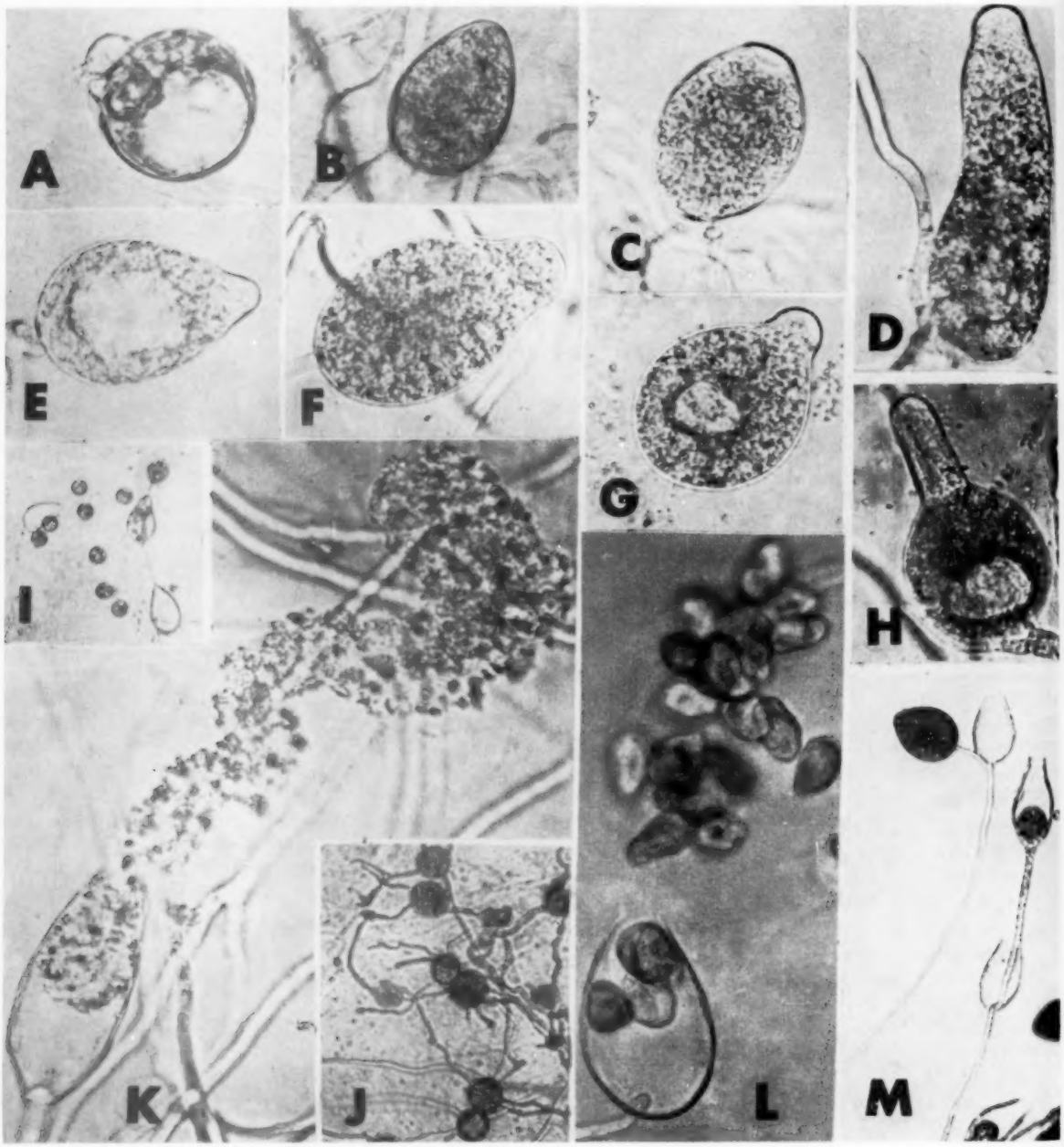

Fig. 1. Various structures of the soybean *Phytophthora*. **A)** oogonium with paragynous antheridium (560×); **B–D)** inconspicuously papillate sporangia (560×); **E–H)** conspicuously papillate sporangia (560×); **I)** chain of resting sporangia and zoospores (125×); **J)** intercalary sporangia (125×); **K)** abortive sporangium (560×); **L)** sporangium that germinated by zoospores (560×); **M)** sporangia (center) appearing to proliferate but actually same as sporangia on left.

TABLE 2.—*The effect of different-aged cultures of the soybean Phytophthora and different batches of Difco Lima-bean agar (DLB) on the range and mean sporangial size and the type of sporangial papillation*

Media	Age of culture[a] (days)	Range (μ)[b]	Mean (μ)[b]	Per cent sporangia with papillae: Inconspicuous	Per cent sporangia with papillae: Conspicuous
New DLB	4.0	55×44–116×55	88×51	100	0
New DLB	4.5	66×33–110×66	80×55	80	20
New DLB	5.5	66×38– 88×60	77×55	80	20
5-yr.-old DLB	4.0	60×38–116×59	88×51	80	20
5-yr.-old DLB	5.5	55×33– 77×44	66×38	100	0
5-yr.-old DLB[c]	18.0	22×16– 66×44	53×38	22	78
5-yr.-old DLB[c]	69.0	16×11– 44×33	32×23	78	22
24-yr.-old DLB	4.0	38×33–110×59	65×33	100	0
24-yr.-old DLB	5.5	38×33– 77×33	55×33	100	0
Distilled water	4.0	22×16– 55×38	29×29	100	0

[a] Cultures were maintained at room temperature.
[b] Based on a total of 50 sporangia from 4 plates.
[c] Separate tests from the others.

germination. Except when diseased tissues were used, no sporangia developed in tap water or Difco Lima-bean agar prepared with tap water. Again, except when diseased tissues were used, the sporangia in distilled water usually aborted whereas the sporangia in Difco Lima-bean agar prepared with distilled water germinated with the formation of zoospores. In the case of diseased tissues, sporangia developed on all media, and both abortive sporangia and zoospore type germination were common. When PDA was used as the source of the fungus, conspicuously less sporangia developed on the Lima-bean agar prepared with distilled water than when other sources were used. The corn, Lima-bean, and soybean agar sources had identical effects on all of the media.

DISCUSSION.—The shapes, sizes, and papillation of sporangia of the species of *Phytophthora* inciting root and stem rot of soybean were quite variable, and the ranges overlapped those of many of the *Phytophthora* spp. with paragynous antheridia. The ranges of oospore size also overlapped those of these species. If the soybean *Phytophthora* should be cultured under various conditions, it might appear that several distinct species were present. Stamps (6) studied the variation in a strain of *P. cactorum* by using single-zoospore isolates. One zoospore gave rise to a range of colony types differing in growth rate and morphology of hyphae; oogonial diameter, sporangial length, and shape and frequency were also variable. The investigation was important since there was no doubt concerning the relationship of the various forms. If the various colonies had not been known to arise from a single zoospore, it might have been concluded that there were several distinct species present.

It appears from these studies that the *Phytophthora* spp. with paragynous antheridia should all be studied in more detail to obtain better taxonomic criteria for species identification.

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ETIOLOGY OF THE PHYTOPHTHORA DISEASE OF SOYBEANS[1]

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SUMMARY

The incitant of root and stem rot of soybean was identified as a *Phytophthora* species by placing diseased tissues on a medium consisting of 2.3 g/l Difco Lima-bean agar. When the pathogen was present, sporangia developed and zoospores were produced within 48 hours. Recovery of the pathogen from field soil, as determined by plant assay, was low after the soil was exposed to dry conditions, but was conspicuously higher after prolonged wet conditions. Viable *Phytophthora* was found in soil mixed in seed samples by harvesting procedures. Soil can be an important means of spread of the pathogen within and between fields. Sporangia and zoospores may be important forms in which the pathogen is disseminated within fields. Abundant sporangia and zoospores developed on flooded diseased soybean plants in soil; some Harosoy soybean plants in steamed soil infested by this flood water became diseased, demonstrating the presence of the pathogen in the water. The pathogen was isolated from immature seeds taken from pods within an advancing margin of rot of stems or from stem lesions, but was isolated from mature seeds only after a two-month cold-wet treatment. The presence of other root pathogens influenced the severity of *Phytophthora* root and stem rot in steamed soil; total kill was significantly reduced with certain combinations of *Rhizoctonia solani* and *Phytophthora* or *Fusarium roseum* and *Phytophthora*. The etiological aspects studied and correlated to field conditions might be applied in future control programs.

INTRODUCTION.—In 1951, a new root and stem rot of soybean was observed in one county in Northwestern Ohio; in 1955, Suhovecky (9) reported that a species of *Phytophthora* was the incitant. *Phytophthora* on soybean has also been reported in Illinois and North Carolina (4, 8). By 1957, this root rot of soybean had been reported in all of the major soybean-producing areas of Ohio. Schmitthenner (6) estimated annual losses in Ohio from *Phytophthora* root and stem rot of soybean to be about $1,500,000. Several soybean varieties are susceptible to this *Phytophthora*; some are resistant, but no present varieties are known to be immune (7). The etiological aspects of the root and stem rot of soybean as correlated to field conditions should have application to an understanding of the pathogen life history in the field as well as to control measures.

The morphology of the soybean *Phytophthora* has been described by Suhovecky (9), Herr (2), Kaufmann and Gerdemann (4), Klein (5), and Skotland (8). Symptoms of the soybean root and stem rot incited by a species of *Phytophthora* have been discussed in detail by Herr (2), Kaufmann and Gerdemann (4), Skotland (8), and Suhovecky (9).

Herr (2) and Skotland (8) reported observing oospores in soybean plant tissue. Herr (2) suggested that the pathogen may overwinter as oospores in diseased soybean tissues. Suhovecky (9) reported that, in combination with *Fusarium* in steamed soil, the soybean *Phytophthora* resulted in significantly less disease than did *Phytophthora* alone. He further reported that less disease resulted in untreated field soil infested with *Phytophthora* than in steamed soil thus infested.

MATERIALS AND METHODS.—A rapid and easy technique was developed for isolating and recognizing the *Phytophthora* species that incites root and stem rot of soybeans. Diseased soybean tissues were placed in a sieve fitted on a 600-ml beaker, one drop of Tween-20 was added, and tap water was run over the tissues for 20 minutes to 2 hours. Tween-20, a wetting agent, was used to loosen surface materials on the diseased tissue. The washed diseased tissues were placed on 2.3 g per liter of Difco Lima-bean agar in Petri plates kept at room temperature. Abundant sporangia developed and zoospores were consistently produced within 24–48 hours, even in the presence of contamination by other fungi or bacteria. In this study, plants were considered diseased by *Phytophthora* if the fungus could be associated with diseased tissues by this technique.

RESULTS.—*Survival of the pathogen in soil.*—The moisture history of soil affected the active *Phytophthora* inoculum as determined by recovery of the pathogen based on a plant-assay technique. Field soil, collected at various intervals, was placed in glazed porcelain crocks in the greenhouse, and 25 Harosoy soybean seeds were planted in each crock. Data were taken 3 weeks after planting. In Table 1 are time of collection, condition of the soil at collection, and percentage of *Phytophthora*-diseased plants. This percentage was very low in soil exposed to dry conditions before planting, and conspicuously higher in soil exposed to prolonged wet conditions before planting.

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TABLE 1.—*Percentage of Phytophthora-diseased Harosoy soybeans planted in field soil collected at various intervals during the year*

Date collected	Condition of soil when collected	Per cent plants diseased
May 1957	wet	90
August 1957	dry	5
November 1957	wet	38
January 1958	frozen (wet)	72
April 1958	wet	85

The effect of various temperature and moisture treatments on recovery of the pathogen from the soil as determined by a plant-assay technique, was investigated. Uninfested field soil, collected dry and stored dry for 8 months, and steamed soil and field soil, infested with diseased tissues containing oospores of the pathogen, were all placed in glass jars and air-dried for 1 month. Portions of each dry soil series were set aside as soil controls, and other portions were saturated with water and treated. One portion of each soil was treated for 3 months by alternately freezing 2 weeks and thawing 1 week; another portion was kept 3 months in the 25°C constant-temperature room; a third portion was, for 3 months, alternately frozen 2 weeks and thawed 1 week, and then kept 3 months in the 25°C constant-temperature room; and the fourth portion was kept 6 months in the 25°C constant-temperature room. After treatment, the jars were placed in the greenhouse and 5 Harosoy soybean seeds were planted in each jar of soil. Three weeks after planting, the number of *Phytophthora*-diseased plants was recorded. In Table 2 are the percentages of *Phytophthora*-diseased plants in the various soils. The percentage of diseased plants increased with the length of the wet period of the soil before planting.

Development of sporangia on diseased plants.—To determine if sporangia and zoospores could be important forms in which the pathogen might be spread within fields, observations were made on sporangial development on diseased Harosoy soybean seedlings growing in steamed soil infested with the pathogen and in soil collected from fields containing *Phytophthora*-diseased plants. In all cases sporangia were found on the hypocotyl of dying seedlings that had just emerged.

In another experiment, one-month-old diseased Harosoy soybean plants growing in steamed soil infested with the pathogen and in field soil were flooded with tap water for 48 hours and 120 hours. Abundant sporangia developed on the hypocotyls within 48 hours, and after 120 hours were still developing in both types of soil; zoospores were abundant. Some of the flood water from the field soil was carefully decanted and used to wet steamed soil; steamed soil wetted with tap water was the control. Harosoy soybean seeds were planted in both soils immediately after the wetting. At 3 weeks, no plants were diseased in the steamed soil wetted with tap water, but 5 plants in various areas of the soil wetted with the flood water were dying, and 35% of the plants were diseased. Since the flood water was carefully decanted, the probable forms of inoculum were external mycelium, sporangia, and zoospores.

Spread of the pathogen on pods and in soil.—The possibility was investigated that the pathogen was seed-borne. Pods were collected from soybean plants with various stem symptoms incited by the root and stem rot *Phytophthora*. The usual *Phytophthora* disease symptom on soybean in Ohio is the root-rot phase, with an advancing margin of stem rot extending from the roots; the rot encircles the stem. A stem-lesion phase, common in fields throughout Ohio in 1957, was observed by Schmitthenner (personal communication). The lesion is a reddish lesion that appears to originate in the roots. The lesions are sharply delimited and characteristically depressed, and in general do not encircle the stem. The species of *Phytophthora* that incites root and stem rot of soybean was isolated from some of these lesions; this lesion symptom was later observed in the greenhouse when soybean seeds were planted in infested steamed soil. Fig. 1 is a photograph of soybean plants showing stem symptoms incited by the soybean *Phytophthora*.

Pods containing immature and mature seeds were collected from within *Phytophthora*-diseased stem areas of soybeans. Seeds were carefully removed from the pods, placed on 2.3 g per liter Difco Lima-bean agar, and the pathogen grew out of some immature seeds taken from pods within the advancing margin of rot or from lesions. *Phytophthora* did not grow out of mature seeds in any case, but oospores were observed on the seed coats of some seeds. The pathogen grew from seeds of Harosoy soybean showing the advancing margin of rot, and from seeds of CX187 variety, H38341 variety, CX171 variety, and Lincoln soybean, all having the lesion symptom.

Thirty-six immature seeds were inoculated with the pathogen in the greenhouse by carefully opening pods still attached to the plants and placing the inoculum on the seed farthest from the peduncle, on the side of the seed away from the other seeds. The pods were then closed and wrapped with Scotch tape. Two days after inoculation of the seeds, one pod was removed from varieties U1-5, H20771, Clark, and C1117, and

TABLE 2.—*Mean percentage of Phytophthora-diseased Harosoy soybeans planted in soil incubated at various moisture levels for various periods after air-drying for 1 month*

Treatment	Per cent of diseased plants in soil[a]			
	Steamed, uninfested	Field, uninfested	Steamed, infested	Field, infested
None	0	5	5	5
3 months cold-wet	0	22	12	26
3 months warm-wet	0	11	22	22
6 months warm-wet	0	84	88	84
3 months cold-wet; 3 months warm-wet	0	100	100	74

[a] Based on 12 replicates.

the seeds were placed on 2.3 g/l Difco Lima-bean agar. The inoculum was removed from the inoculated seeds, and these seeds were thoroughly washed to remove surface mycelium. The seeds were still immature, and the pathogen grew from all inoculated seeds as well as from the seeds next to them. The *Phytophthora* did not grow from 27 inoculated seeds that were mature 7 and 14 days after inoculation, although oospores were observed on seed coats of all inoculated seeds. The remaining pods were placed in a Manila envelope and left buried 1 ft. deep in outside soil from December, 1957, to February, 1958. The pods were then dug up, seeds were placed on 2.3 g/l Difco Lima-bean agar or distilled water, and the pathogen grew from 2 of the inoculated seeds. Oospores were observed on seed coats of all inoculated seeds, but no oospores were observed to have germinated. The pathogen grew from the embryo but not from the seed coat. According to these data, seeds could be important in spreading the pathogen between fields; as the seed matures the pathogen may become dormant but still viable.

The importance of soil in spread of the pathogen was investigated, since many particles of soil similar in size to soybean seeds were found in seed samples. A soil sample was separated from a soybean seed sample, placed in glass jars, saturated with water, and Harosoy soybean seeds were planted in one portion immediately and in two other portions after the soil had been left 2 months in the 4.5°C constant-temperature room. Diseased plants were observed 2 weeks after planting, but only in soil that had been saturated and left 2 months in the 25°C constant-temperature room. From these data it is inferred that soil mixed with seed could be important in spreading the pathogen between fields.

Interactions of the soybean Phytophthora with other root pathogens.—To study the effect of the presence of other root pathogens on the severity of *Phytophthora* root and stem rot of soybeans, 17-day-old PDA (potato-dextrose agar) slant cultures of the soybean *Phytophthora*, *Rhizoctonia solani* Kuhn. from alfalfa, and *Fusarium roseum* Snyd. & Hans. from corn were chopped into small pieces and mixed individually and in various combinations in steamed soil. Each treatment consisted of 4 replicates of 5 plants each. In about 3 weeks, 80% of the control plants (in the soil with a high level of *Phytophthora* alone) were killed, and the data were taken. The mean number of dead plants for each treatment is recorded in Table 3. A high level of a fungus consisted of one PDA culture mixed with steamed soil from four 2-in. clay pots; a

Fig. 1. Soybean plants with stem symptoms incited by the soybean *Phytophthora*. The plant on the left has an advancing margin of rot extending from the roots and encircling the stem. The plant on the right has lesions extending from the roots but not encircling the stem. The plant in the center has a more extreme lesion symptom.

TABLE 3.—*Mean number of dead Harosoy soybean plants in steamed soil infested with fungi in various combinations (inoculum 17 days old)*

Other fungi	*Phytophthora*		
	High[a]	Low[a]	None
High *Rhizoctonia*	3.00	2.75	1.00
Low *Rhizoctonia*	2.75	2.50	1.50
High *Fusarium*	2.75	2.00	1.00
Low *Fusarium*	2.25	3.00	0.25
Control	4.25	3.50	0.00
LSD[b] at 5% level = 0.80			
LSD[b] at 1% level = 1.09			

[a] High or low refers to the inoculum level.
[b] LSD based on statistical interaction: levels of *Phytophthora* × other fungi.

low level of a fungus consisted of one PDA culture mixed with steamed soil from eight 2-in. clay pots. There was significantly less kill with certain combinations of *Phytophthora* and *Fusarium*, or *Phytophthora* and *Rhizoctonia*. Only 10% of the dead plants were killed before emergence.

Observations were also made on the surviving plants. Except for the series of *Fusarium* alone and the combination of a low level of *Phytophthora* and a high level of *Fusarium*, the plants were distinctly stunted as compared with plants in steamed-soil checks. Delayed emergence was very evident in all soil infested with *Rhizoctonia* alone or in combination; when the plants in the *Rhizoctonia* series did emerge, many seedlings were abnormal, and some stem tips or the embryo root tips appeared to have been destroyed, although lateral buds or adventitious roots developed. *Rhizoctonia* lesions were observed on the hypocotyl in nearly all of the *Rhizoctonia* series. In all series in which the soil had been infested with *Phytophthora* alone or in combination, *Phytophthora* was found in all diseased plants.

In preliminary experiments with *Pythium ultimum* Trow isolated from alsike clover, the mean total kill was not statistically significant when compared with the *Phytophthora* control.

DISCUSSION.—The incidence of *Phytophthora* disease on soybean assay plants was low in soil that had been air-dried for an extended period before planting, and was conspicuously higher after prolonged wet periods before planting. Since Blackwell (1) reported that mycelium, conidia, resting conidia, chlamydospores, and oospores of *Phytophthora cactorum*, if not completely desiccated, could remain dormant for months, the decrease in recovery of the pathogen during dry periods could be attributed to the formation of resting forms that were inactive, though viable. During prolonged wet periods, dormant forms may become active again. No field evidence was available to substantiate any of these hypotheses. More detailed studies of this type would be very useful in determining inoculum potential and forms of survival of the pathogen in the field.

Sporangia and zoospores could be very important in the spread of the pathogen within fields. Results of this type of study might be applied in future control procedures concerning drainage since, during prolonged wet conditions, certain areas of soybean fields are commonly flooded for several days. Also, the data are in agreement with those of Hickman (3), who found that, in wet soil, zoosporangia of *P. fragariae* developed naturally on the surface of recently diseased strawberry roots. He thought that zoospores could be important in the spread of the pathogen, especially in surface drainage water.

The fact that the pathogen was found to be seed-borne could be important in the spread of the pathogen between fields, and therefore in control of the disease. More data are needed concerning this aspect and will be important in possible seed-registration and seed-treatment programs. Field soil, inadvertently included in seed samples as a result of harvesting procedures, contained viable soybean *Phytophthora*. Thus the fact that the pathogen could be spread between fields in soil mixed with seeds might be applied in future seed registration and certification material.

There was significantly less kill of Harosoy soybean when certain combinations of the soybean *Phytophthora* and *Fusarium* or *Rhizoctonia* were used. Whether the interactions of the fungi were antagonistic or whether the metabolism of the host was altered in some manner is still unknown. The results of this type of study could be applied in future rotation and in fungicide programs since both of these programs could affect the microflora balance.

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GENETICS OF COCHLIOBOLUS HETEROSTROPHUS. IV. A MUTANT GENE THAT PREVENTS PERITHECIAL FORMATION[1]

R. R. Nelson[2]

SUMMARY

Certain *A* monoascosporic isolates obtained from a cross of 2 wild-type conidial strains of *Cochliobolus heterostrophus* exhibit complete mating inhibition to the compatible *a* parental strain and to about half of the *a* strains isolated from nature. Studies on the segregation of ascospore isolates from crosses of normal and inhibitor strains indicate that mating-type inhibition is controlled by a single recessive gene, "i," inherited independently of the locus for compatibility. The inhibitor gene blocks the formation of protoperithecia. Adding a number of different kinds and quantities of growth factors to supporting media failed to induce perithecial formation. The widespread occurrence of the mutant gene in wild-type *a* strains, and its absence in wild-type *A* strains, indicate that its origin was early in the evolution of the species. The discovery of the mutant "i" gene offers further evidence of the complexity of the sexual mechanism in *C. heterostrophus*.

In recent years, a number of investigations on the genetics of sexual reproduction in fungi have contributed important evidence as to the complex nature of the sexual mechanism. Such information has not minimized the importance of the compatibility locus, but has pointed out that this locus is only a single link in the reproductive cycle.

The demonstration of genetic factors reported to block the completion of the sexual process at various stages has contributed important information concerning the origin of heterothallism in the fungi. It has been demonstrated that genetic blocks may occur at 1) formation of the protoperithecium, 2) plasmogamy, 3) karyogamy, and 4) reduction division (1, 2, 7, 8, 9, 10). Previous studies on the genetics of sexual reproduction in *Cochliobolus heterostrophus* Drechsl. have demonstrated the presence of genetic factors that block ascus formation (6) and ascospore production (5). Recent crosses of wild-type conidial strains of *C. heterostrophus* have yielded monoascosporic isolates that exhibit complete mating inhibition to both parental isolates and to certain wild-type isolates of the opposite compatibility group. The results of studies with these isolates are reported herein.

MATERIALS AND METHODS.—The techniques employed were similar to those previously described (3). Compatible isolates of *C. heterostrophus* were mated by placing small pieces of mycelium on opposite sides of a section of sterile corn leaf, about $1\frac{1}{2} \times \frac{1}{2}$ in., in the center of a Petri plate containing Sach's nutrient agar. All matings were incubated at 24°C.

Eighteen monoascosporic isolates were obtained from a cross of an *A* strain, designated T1, isolated from diseased corn leaves collected in western North Carolina, and an *a* conidial strain, designated 54H, from Colombia, South America. Both isolates were considered "weak" representatives of their compatibility groups, as measured by relative degree of intergroup compatibility (4). Whenever paired, T1 and 54H developed few perithecia, with relatively poor ascus and ascospore production. The compatibility reaction of the 18 ascosporic isolates was determined by mating each isolate to both parental strains, T1 and 54H, and to *A* and *a* tester strains. Three of the 7 *A* isolates obtained failed to mate with the *a* parental strain (54H), although all 7 of the *A* isolates were fertile with the *a* tester. Each of the 11 *a* isolates mated with the *A* parental strain and the *A* tester. All subsequent pairings of the 3 *A* isolates and 54H (*a*) also were unfertile. The original parents, T1 and 54H, were mated again, and 12 ascospores were isolated. Four of the 6 *A* isolates failed to mate with 54H (*a*), but were fertile with the *a* tester, designated W3. The 6 *a* isolates were again fertile with both the *A* parental isolate (T1) and the *A* tester. The results summarized in this paper are concerned only with studies of the ascospore progenies obtained from the initial mating. Since the 3 *A* isolates behaved similarly in all studies, the results presented are concerned with only 1 of the isolates, hereafter designated isolate 4.

RESULTS.—Isolate 4 was paired with 27 *a* conidial strains representing a wide geographic and genetic base. Repeated testing showed that isolate 4 was compatible with 12 of the strains whenever paired, and was never compatible with the remaining 15 strains. There was no apparent relationship between the geographic source of the strains and their reaction with isolate 4. However, when the relative degree of compatibility of the 27 strains was considered, it was found that 13 of the 15 strains that failed to mate with isolate 4 were considered to be "weak" strains, as measured by the relative frequency with which isolates failed to cross with known compatible isolates (4).

The reaction of 84 ascospore progenies isolated from a cross of isolate 4 and a compatible *a* conidial strain, W3, was studied by pairing each of the monoascosporic isolates with both parental strains, 4 and W3,

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TABLE 1.—*Production of mature perithecia of Cochliobolus heterostrophus in matings of 6 monoascosporic isolates and 4 monoconidial isolates*

Compatibility group	Isolate	Mating reaction* in crosses of indicated isolates			
		A		*a*	
		T1	4	W3	54H
A	1	—	—	F	I
	2	—	—	F	I
	3	—	—	F	I
	4	—	—	F	F
	5	—	—	F	F
	6	—	—	F	F
a	7	F	F	—	—
	8	F	F	—	—
	9	F	I	—	—
	10	F	I	—	—

*— denotes perithecia not produced in pairings of isolates of same compatibility group; F denotes mature perithecia produced in pairings of isolates of opposite compatibility groups; I denotes perithecia not produced in pairings of isolates of opposite compatibility groups.

and to the original strains, T1 and 54H. Of the total, 44 belonged to compatibility group *A*, and 40 to *a*. Twenty-five of the 44 *A* isolates were fertile with W3 and 54H, whereas 19 were fertile only with W3. All 40 of the *a* isolates were fertile with T1, but only 21 were fertile with isolate 4. These results suggested that the mechanism preventing the formation of perithecia between compatible strains was controlled by a single gene, independent of the locus for compatibility. To test this possibility further, 6 *A* and 4 *a* of the 84 ascospore isolates obtained from the cross of 4 × W3 were mated with the following isolates: the 6 *A* strains to W3 and 54H (if not inhibited); the 4 *a* strains to T1 and 4 (if not inhibited) (Table 1). Ascospores isolated from these crosses were then mated back to T1, 4, W3, and 54H. As part of the postulation, isolates that had demonstrated inhibition in previous crosses were considered to have a recessive gene, designated i, for inhibition. Conversely, isolates that had shown no inhibition in compatible crosses were considered to carry a dominant factor, I. The results are summarized in Table 2.

These studies demonstrated that the failure of compatible strains to produce perithecia when paired is governed by a single gene locus inherited independently of the locus for compatibility.

Attempts to produce perithecia in pairings of inhibitor strains by the use of a number of media varying in nutrients and in pH were not successful. Twenty amino acids and 25 vitamins were tested singly and in combinations at 2 levels. Seven carbon sources and 4 nitrogen sources were also tested at 2 concentrations.

DISCUSSION.—The fact that monoconidial and monoascosporic isolates of *C. heterostrophus* normally do not produce protoperithecia when selfed indicates that a stimulus is required to initiate perithecial formation in paired cultures. The failure of paired strains of opposite compatibility to produce protoperithecia represents a basic block in the reproductive cycle.

It is of considerable importance to note that the "i" factor for inhibition is not present in any of the more than 40 *A* strains obtained from nature. This is confirmed in that all of the *a* strains carrying the "i" factor mate with all *A* strains to produce fertile perithecia. The inability to recover the "i" factor from *A* strains suggests that the sexual stage of the fungus is not common in nature, although representative strains of both compatibility groups have been recovered several times from the same field of corn, and once from the same lesion.

The mechanism by which the inhibitor gene blocks perithecial formation is not fully understood. The negative results obtained with the addition of numerous vitamins, amino acids, and carbon and nitrogen sources suggest that a deficiency for a required growth factor is probably not involved.

In previous studies with *C. heterostrophus*, monoascosporic isolates were obtained that produced normal-appearing perithecia when selfed, although the perithecia contained no asci (6). The fact that sterile perithecia are produced in selfed cultures of these variants and in paired cultures of "sterile perithecia" strains and normal strains, coupled with the nearly complete absence of ascogenous hyphae in the sterile perithecia, indicates that a separate genetic mechanism governs the initiation of perithecia. Therefore, it is postulated that the "i" gene functions in such a way as to inhibit that process.

The fact that the "i" factor is present in about half of the wild-type *a* strains obtained from diverse geographic areas, but absent in wild-type *A* strains, indicates that the establishment of the "i" locus occurred early in the evolution of the species. The presence of

TABLE 2.—*Segregation for compatibility and mating type inhibition in progenies of Cochliobolus heterostrophus obtained from pairing inhibited and normal isolates*

Isolates	Genotype of isolates	Genotype of isolate paired with	No. of spores isolated	Genotype of ascospores isolated			
				AI	Ai	aI	ai
1	Ai	aI	50	16	13	10	11
2	Ai	aI	37	7	10	12	8
3	Ai	aI	33	9	7	8	9
4	AI	aI	26	14	0	12	0
		ai	14	3	3	2	4
5	AI	aI	32	15	0	17	0
		ai	13	2	3	5	3
6	AI	aI	28	15	0	13	0
		ai	29	5	9	9	6
7	aI	AI	33	18	0	15	0
		ai	27	7	8	7	5
8	aI	AI	42	20	0	22	0
		Ai	38	7	10	12	9
9	ai	AI	29	6	7	7	9
10	ai	AI	25	8	5	5	7
Totals:							
AI × aI				82	0	79	0
AI × ai				24	27	28	29
Ai × aI				46	48	49	41

the "i" locus also indicates a basic genetic difference among strains of the *a* compatibility group.

A more thorough understanding of the number and function of genes that block the completion of sexual reproduction should provide an important insight into the evolution of heterothallic species.

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PRODUCTION OF NEW RACES OF PUCCINIA GRAMINIS VAR. TRITICI BY VEGETATIVE FUSION[1]

G. H. Bridgmon[2]

SUMMARY

Urediospores of *Puccinia graminis* var. *tritici* races that were distinct in spore color and pathogenicity were mixed manually and injected into wheat plants in the late boot stage of growth. The mixtures produced at least 16 races that differed from the parents in color and pathogenicity.

Recent studies (1, 2, 3, 5, 6) have shown that new races of rust arise by genetic exchange through fusions of hyphae or germtubes. The present studies are a verification and extension of work with *Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Shear, Ball, Jacks, & Stakman. Pertinent literature has been reviewed by Nelson et al (3) and Watson (6).

Materials and methods.—Single-spore isolates of races 9 and 11 (orange urediospores) and race 121 (gray-brown urediospores) of *P. graminis* var. *tritici* were used in mixtures. The infection types of the races were first determined several times on the standard differential wheats (4) plus Lee, Bowie, and Kenya Farmer. Selkirk, K-360-H, S-131, McMurachy, and Golden Ball were used as supplementary differential wheat varieties when a more extensive analysis of racial purity was desired.

About equal quantities of urediospores of each race were mixed, suspended in distilled water, and immediately injected (using a Vineland Poultry hypodermic syringe with a 10-ml plastic reservoir) into the boots of wheat plants a few days before the heads were due to emerge. Enough inoculum was injected to fill the boot (0.1–0.6 ml of liquid). About 300 adult wheat plants were inoculated 4 times. Plants of the varieties Khapli or Lee were usually used because these wheats were resistant to most races being studied in the greenhouses and it was believed that contamination could thus be kept at a minimum. The small size of the rust pustules on these wheats also made isolation of the red uredia easier. Pure cultures of each parental race, as well as distilled water alone, were injected into plants as controls.

Inoculated plants were held in the greenhouse without being placed in moist chambers. About 20 days after inoculation the plants were examined for red uredia, which were transferred to Little Club seedlings by removing the urediospores with a small, flat needle and incubated in a moist chamber. The study was carried out in late winter and early spring to reduce the possibility of contamination from outside sources. The infection types of each new isolate derived from one or more mixtures were tested from 4 to 12 times before tests were discontinued.

Experimental results.—At least 15 different races or subraces with red urediospores were isolated, most of them several times, from 4 separate mixtures of

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TABLE 1.—*Reactions of differential varieties of wheat to stem rust races 11 (orange) and 121 (gray-brown) and to new variants obtained from mixing these races*

Wheat varieties	Mean infection types[a] produced						
	Parent races		New red races and subraces				
	Orange race 11	Gray-brown race 121	NR1	NR2	11a	32a	59c
Little Club	4	4	4	4	4	4	4
Marquis	4–	3	3	X	4–	4–	2
Kota	4–	0	3+	2+3–	3+	3+	2
Reliance	4–	0	3+[cn]	3	3+	3+	0
Lee	1	1	0;	0;1[b]	1–	1–	1
Arnautka	3	0;1	0;1	0;1	3	X	0;1
Mindum	3	0;1	0;	0;1	3	X	0;1
Spelmar	3	0;1	0;	0;1	3	X	0;1
Kubanka	4–	3	1–	0;1	3+	X+	X
Einkorn	3+	3	0;	0;1	3–	3–	3–
Vernal	0;1	3	0;1	0;1	0;	0;	0;
Acme	3+	3	0;1	0;1	3	X	4–
Khapli	0;1	0;1	1+	0;1	0;	0;	1–
Bowie	0;1	0;1	2[c]	X	4–	4–	2[d]
Kenya Farmer (K338)	0	0	0;	0;	0;	0;	0;

[a] 0 = immune, 0; = flecks, 1 = very resistant, 2 = moderately resistant, 3 = moderately susceptible, 4 = very susceptible, X = heterogeneous (4). + and – indicate variation within infection types. c and n mean chlorosis and necrosis, respectively. Stability of infection types tested 4—12 times each.
[b] Type 0; to type 1 occurred consistently on each plant of the variety.
[c] A tendency to X-type reaction sometimes noted.
[d] Flecks and 1-type reaction occasionally noted.

races 11 and 121, with orange and gray-brown urediospores, respectively. Five of these isolates represented previously undescribed races or subraces (Table 1). The isolate designated 59c differed from previously known isolates of race 59 because it produced infection type 2 on Kota. The new races NR 1 and NR 2 resembled each other except for relatively minor differences on Marquis and Bowie. Isolates designated 11a and 32a were similar to races 11 and 32 except for increased virulence on Bowie wheat. In addition to races 11a and 32a, 8 isolates were obtained that were intermediate between them. These isolates, unlike any described (4) to date, produced infection types X and 3 in various combinations on the durums, but were otherwise similar to races 11a and 32a. Two other isolates were also obtained: the first was tentatively identified as race 111 and the second produced only flecks on Little Club wheat. Neither isolate was completely identified, because the cultures were lost. All of the derived races listed in Table 1, as well as the 8 approaching 11a and 32a in pathogenicity, were characterized by being more virulent on Bowie wheat than either of the parent races. Spore color and pathogenicity of the derived races apparently were stable, although minor variations occurred in pathogenicity of NR 1 and 59c on Bowie.

TABLE 2.—*Reactions of differential varieties of wheat to stem rust races 11 (orange) and 121 (gray-brown) and to new variants obtained from mixing these races.*

Wheat varieties	Mean infection types[a] produced		
	Parent races		New red race
	Orange race 9	Gray-brown race 121	15
Little Club	4	4	4
Marquis	4–	3	4–
Kota	4–	0	3+
Reliance	0	0	4–
Lee	0;1	1	12
Arnautka	3	0;1	3+
Mindum	3	0;1	4–
Spelmar	3	0;1	3+
Kubanka	3	3	4–
Einkorn	3–	3	3
Vernal	3–	3	3
Acme	4–	3	4–
Khapli	0;1	0;1	1+n[b]
Bowie	0;1	0;1	123c[c]
Kenya Farmer (K338)	0;	0	0;1

[a] 0 = immune, 0; = flecks, 1 = very resistant, 2 = moderately resistant, 3 = moderately susceptible, 4 = very susceptible, X = heterogeneous (4). + and – indicate variation within infection types. c and n mean chlorosis and necrosis, respectively. Stability of infection types tested 4 times.
[b] Some 2 tendency.
[c] Some X tendency.

Essentially the same experiment was made with race 9 (orange urediospores) mixed with gray-brown race 121. With this mixture, only one red race, a biotype of race 15, was isolated (Table 2). This biotype of race 15 characteristically produced infection type 1+, with a heavy amount of necrosis, on Khapli. The race was also more pathogenic on Bowie and Kenya Farmer. Perhaps the most striking characteristic of this new biotype of race 15 was its increased pathogenicity on Reliance, which remained free from rust when inoculated with either of the parent rust cultures.

DISCUSSION AND CONCLUSION.—In the present studies, mixtures of urediospores in aqueous suspen-

sion were hypodermically inoculated into the boots of adult wheat plants in preference to inoculating seedlings. This technique seemed to favor the production of new races, perhaps because of close association of the spores in the boot, or the relatively contaminant-free environment may have been an advantage.

When races 11 and 121 with orange and gray-brown urediospores, respectively, were mixed, at least 15 different red races and biotypes were obtained, which is more than expected from simple nuclear reassortment. It is possible that any or all of the processes of variation suggested by Watson may have been operative: a) nuclear pairing regardless of sex, b) cytoplasmic influences, and c) parasexualism. More work will be needed before the relative importance of these processes can be assessed.

The present work indicates that, when certain races are used in mixtures, unusual biotypes may be formed. Eight of the 15 new races resembled races 11a and 32a except that, on the varieties of *Triticum durum* Desf., they were intermediate between these two races, making it impossible to categorize them taxonomically.

The increased pathogenicity of races derived by fusion of germ tubes or hyphae of different races noted by Nelson et al (3) and Watson (6), was verified in this investigation. An increase in pathogenicity on Bowie was noted in every new race produced, which suggests that the parent races may be heterozygous for genes conditioning virulence on Bowie, with avirulence being dominant, or that they possessed complimentary genes for virulence. Pathogenicity on Reliance also increased when races 9 and 121 were used as parents. Pathogenicity was lower than that of the parent races when NR 1 and NR 2 were inoculated on the durum varieties. Usually, however, each new race approached the pathogenicity of one of the parent races.

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MORPHOGENESIS AND HISTOPATHOLOGY OF LESIONS INDUCED ON CITRUS ROOTS BY RADOPHOLUS SIMILIS[1]

E. P. DuCharme

SUMMARY

Burrowing nematodes, *Radopholus similis*, in the process of becoming established in citrus roots, entered actively growing root tips in the region of cell elongation and root hair production. Roots were penetrated by the lysis of successive cells. As the nematodes progressed through the root, tunnels and cavities were formed in the cortex and stele. All types of parenchyma tissues were attacked. Inside the root the nematodes entered the stele through the passage cells of the endodermis. The phloem-cambium ring, where they accumulated in large numbers, seemed preferred. The phloem-cambium ring was often virtually destroyed in the lesion area, so that the stele was girdled by a nematode-filled cavity separating what was left of the stele from the cortex. Starch disappeared from the cells in and immediately adjoining the lesion area. Wound gum accumulated next to the stele in the invaded area, imparting a tan to amber color in the older portions of the lesion. Cells exposed to the nematode metabolites sometimes became hypertrophied. Hyperplasia and tumor formation from the pericycle occurred when the nematodes passed through the endodermis and became situated next to the pericycle. These pericycle tumors were also attacked by the nematodes.

The biotic association between burrowing nematodes (*Radopholus similis* (Cobb. 1893) Thorne, 1949) and citrus is one of antagonistic symbiosis. Burrowing nematodes, being phytophagous and incapable of independent existence, rely entirely on living roots for food and shelter. These nematodes are normally inside living rootlets, and their activities directly influence tissues and lead to profound changes in the internal morphology of the rootlets. The transformations that occur in the parasitized roots are a manifestation of the interaction arising from the intimate association between the burrowing nematode and host.

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This paper describes the penetration of roots by burrowing nematodes and the changes that occur in the internal morphology of citrus rootlets parasitized by burrowing nematodes in gnotobiotic cultures, i.e., cultures of an organism in an environment in which other species are either known or absent (5).

Goodey (8) did not consider that burrowing nematodes affect plants to produce a gross enlargement of the affected parts. He considered the principal action to be a powerful lysis whereby cell walls and cell contents are destroyed with the production of cavities in the cortex of affected roots. Wart-like thickenings on coffee roots attacked by burrowing nematodes have been noted by Bally and Reydon (1). The presence of small swellings and knots on citrus roots at locations of attack by *R. similis* has been reported by Feder and Feldmesser (7), but no observations were made on the internal condition of the affected roots. The gross appearance of burrowing nematode lesions on citrus roots has been described (6).

METHODS.—Burrowing nematodes and grapefruit seedlings were grown in 2-membered cultures, free of other organisms. Aseptic grapefruit seedlings cultured from seed on sterilized water agar in Petri dishes were inoculated by placing 1–10 adult female burrowing nematodes next to each root tip and incubating 3–50 days at room temperature. The nematodes used were surface disinfected for 15–30 minutes in a 0.001% solution of 8-hydroxyquinolin sulfate. Nematode activities preceding root penetration and the process of root penetration itself were observed directly through a dissecting microscope. Internal transformations of parasitized roots were studied in roots that were killed, fixed in formalin acetic acid and alcohol solution, embedded in paraffin, and cut in cross and long sections 10μ thick. The sectioned material, mounted on slides, was stained with Delafield's haematoxylin or Johansen's quadruple stain.

RESULTS.—Inoculation of grapefruit seedlings was successful with 1 or more adult female burrowing nematodes per seedling. When nematodes were placed next to or on the root tip, they moved about for a while, over the surface of either the agar or the root, and finally located in the region of cell elongation and root hair production, near the root tip. Then the nemas often remained immobile for a time, oriented parallel to the long axis of the root.

Penetration into the root, usually direct, took place within 24–72 hours from the time when the nemas were placed next to or on the root tip, but occasionally 5–6 days elapsed. At times, some of the parasites never penetrated the root, but roamed over the surface of the agar until they died. In delayed penetration the nemas attacked a patch of cells and formed an irregular, shallow, crater-like depression before working into the root. When this occurred, 96 hours elapsed before the parasite burrowed into the cortex. In the early stages of rapid root invasion, there is little or no indication on the root surface where the nema or nemas entered except for a tiny hole with a diameter slightly larger than that of the nematode. Incipient lesions could be detected on the surface of the root 5–7 days after root penetration, by a slight discoloration and splitting of the epidermis over the attacked area. In 15–20 days one or more deep irregular cracks appeared over the lesion cavity, the borders of the lesion were slightly raised, and the area became a darker tan to brown than the healthy portions of the root (Fig. 1).

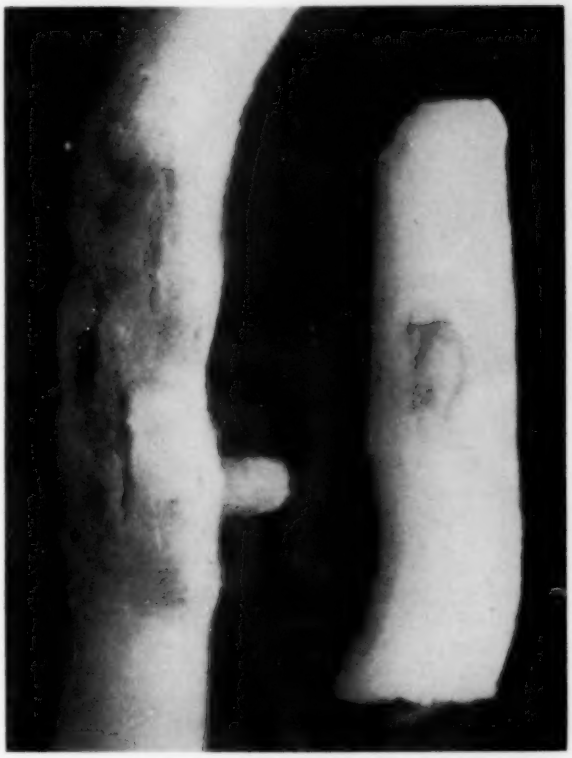


FIG. 1. External appearance of lesions caused by burrowing nematodes on grapefruit roots (×11).

The nematodes penetrated the juvenile epidermis and the underlying cortex parenchyma by apparently dissolving cells. There seemed no mechanical forcing apart of cells; rather, the nematode's forward progress appeared to be accomplished by lysis of successive cells. In effect, the burrowing nematode digests and eats its way through the root tissues. In this manner the female and its progeny form tunnels and irregular cavities housing numerous nematodes (Fig. 2).

When nemas lived more than 3 weeks in the same part of the root, the tunnels and cavities formed an extensive network. The bulk of the root tissues is made up of cortex and is consequently invaded extensively, yet the phloem and cambium ring seem to be the preferred tissues and in time may be completely destroyed in the lesion area, and the tunnels eventually girdle the vascular cylinder (Fig. 3-A).

All types of parenchyma tissues may be attacked and destroyed. The kinds of tissues invaded were nonsuberized epidermis, root hairs, cortex, immature endodermis, passage cells in the endodermis, pericycle,

medullary rays between bundle caps, phloem, cambium, xylem parenchyma, juvenile pith, and the apical meristem, including the derived histogens. The nemas entered the stele through the passage cells in the endodermis, opposite the protoxylem points, and settled in the medullary rays. From there the nemas migrated laterally into the phloem-cambium ring and pericycle. Tissues not attacked were suberized epidermis, exodermis, endodermis, mature xylem, hardened pith, periderm and hardened root cap.

Lysis of the cell walls, and the cell contents of the tissues attacked, accounted for much of the damage to the roots. Starch disappeared from the cells in and immediately surrounding the lesions. Other cells were affected, even though only indirectly exposed to the influence of the burrowing nematodes. The zone of tissues around a lesion from which the starch disappears becomes somewhat clarified or translucent, and the cells may be separated much more easily than in the unaffected portion of the root.

There was a deposition of wound gum in the cortex next to the stele at the site of nematode activity. Wound gum seems to be the only ergastic substance deposited, imparting a tan to amber color in the affected area of the parasitized roots (Fig. 2).

When tissues were exposed by depredations of the burrowing nematodes in monoxenic (two-membered) cultures, there was no formation of wound cork in healthy tissues surrounding the lesion area. Likewise, there was no hardening or thickening of the walls of cells exposed in the lesion cavity. These cells remained moist and turgid. They did not necessarily hypertrophy, although there was a bulging on the exposed side.

After entering the root, nemas usually moved upward but sometimes worked toward the root tip. In the latter case the apical meristem and ground tissues were invaded, and root growth ceased (Fig. 3-B). Healthy root tips taper to a point, but invaded tips sometimes became distorted, because of hyperplasia, and grew into a knob 3–4 mm in diameter. There was no well defined apical point, and terminal growth seemed to take place in all directions. The mass of cells remained undifferentiated except for xylem cells distributed irregularly, singly, or in clusters (Fig. 4-A). The xylem cells were shorter and more truncated than normal ones, and seemed to have lost their polarity. In other cases the tissues in the root tip were virtually destroyed (Fig. 3-B).

Perhaps the most striking response of the plant to burrowing nematodes and their metabolites was the formation of tumors in the pericycle. The pericycle was the only tissue other than the primary apical meristem and derived histogens that was stimulated to

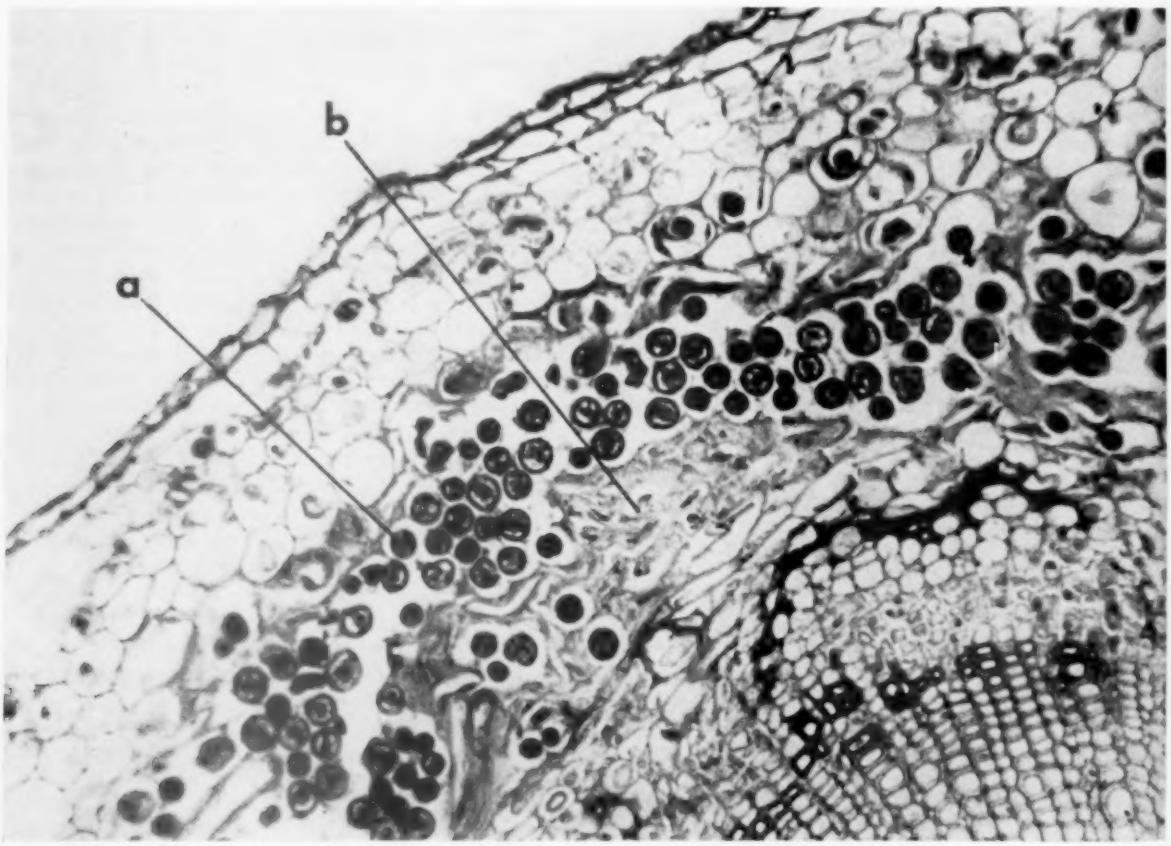


FIG. 2. Cross section of a grapefruit root containing *A*) deposit of wound gum (×260). cross section of burrowing nematodes in a cavity and *B*)

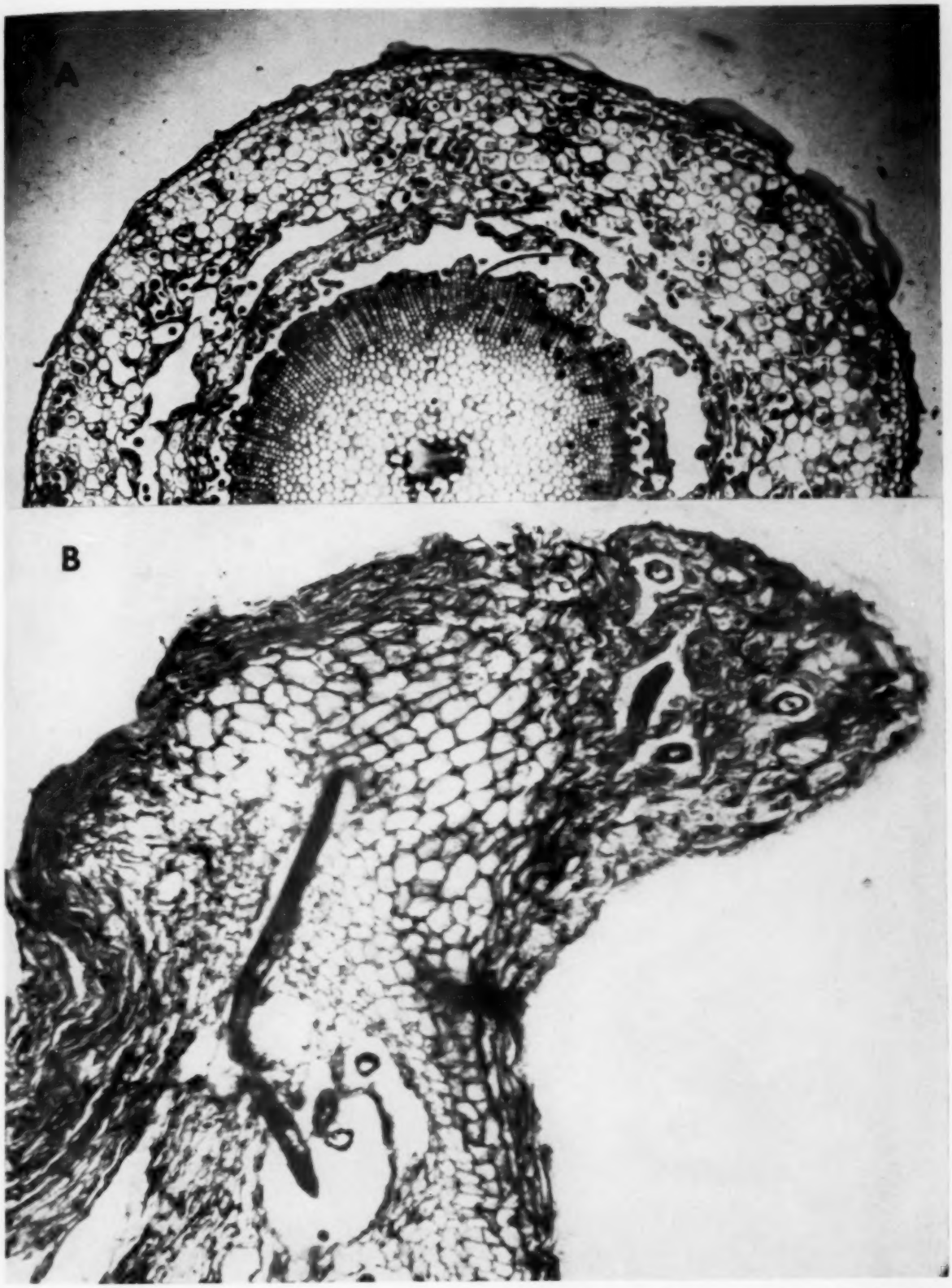
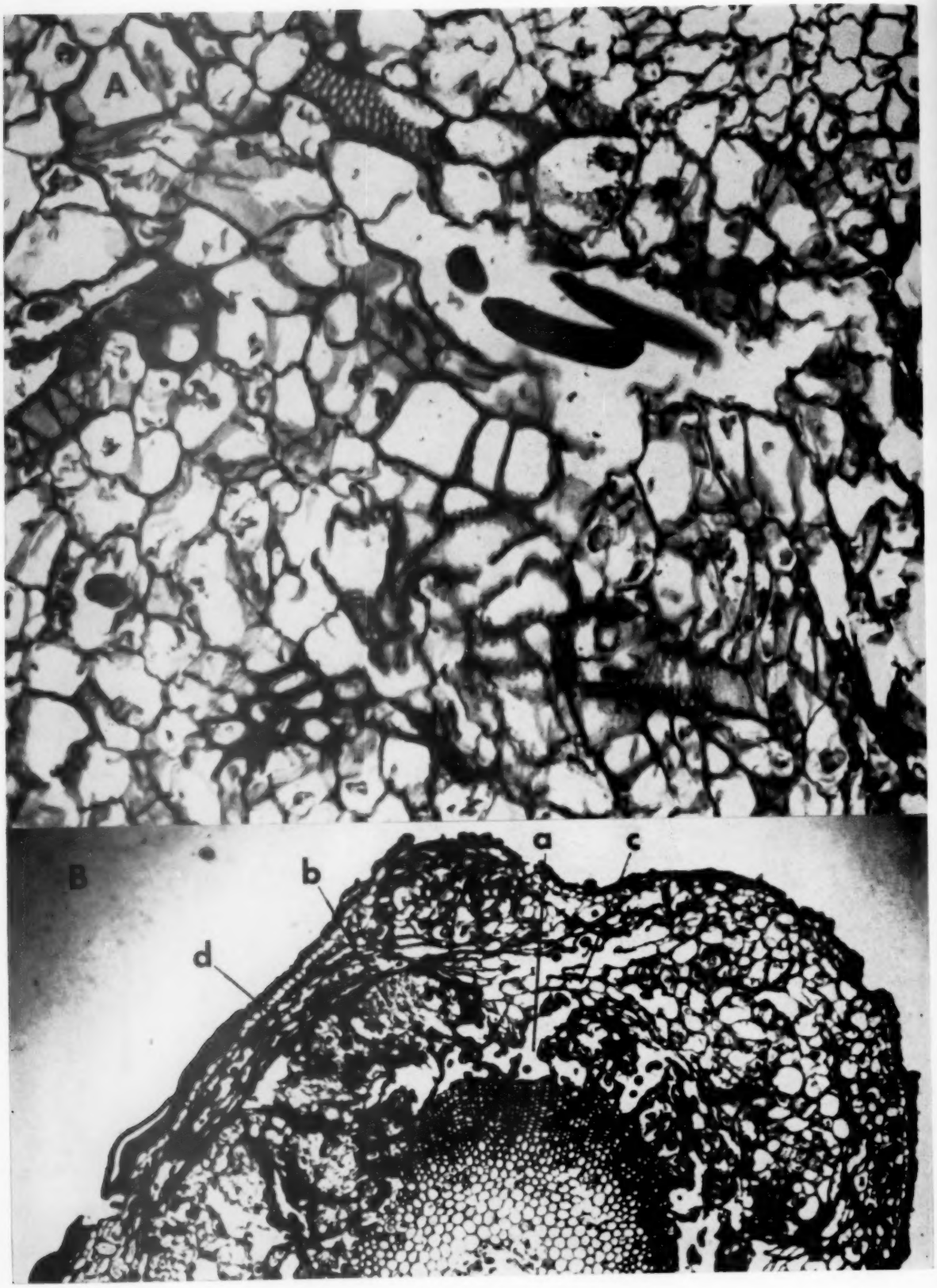


FIG. 3. *A*) Cross section of a grapefruit root in which the phloem-cambium ring has been destroyed by burrowing nematodes (×70). *B*) Longitudinal section through a grapefruit root parasitized by burrowing nematodes (×200).


A
B
a
b
c
d

hyperplastic growth. This morphogenic disturbance in the pericycle occurred only when burrowing nematodes passed through the endodermis and located in the stele.

The first change to take place in the pericycle occured after the parasite passed through the endodermis via the passage cells and fed in the medullary ray tissues in the stele of the primary roots. Initially, there was a hypertrophy of pericycle cells, which became only slightly swollen or increased many times their normal size (Fig. 5-A). The hypertrophied cells neither increased in density of cell contents nor became enriched in any way; instead, the cell contents became diluted. The morphogenic stimulus in other cells leads to hyperplasia. The stimulated pericycle cells divided tangentially, and the volume of the pericycle in the affected bundle increased and formed a discrete tumor made up of cells all more or less alike in size and appearance. They were uninucleate, thin-walled, and generally arranged in rows perpendicular to the long axis of the root; the cytoplasm was finely granular, uniform in density, and finely vacuolate (Fig. 5-B).

These pericyclic tumors were at first limited in width by the medullary rays (Fig. 5-A). Later, as the hyperplastic growth continued, anticlinal as well as periclinal cell division took place in the outer layers of the tumor, the individual tumors eventually coalesced, and the stele became girdled by the much enlarged pericycle (Fig. 4-B). The length of the tumors usually corresponded to the longitudinal distance that the nematodes lay next to the pericycle (Fig. 5-B). The beginnings of hyperplastic growth were evident in the pericycle 10–15 days after initial root invasion, but did not occur as long as the endodermis remained undamaged. The nematodes also fed on and burrowed into the pericycle tumors (Fig. 4-B, 5-B).

Direct mechanical damage to roots from burrowing nematodes is virtually non-existent. There seems to be no violent tearing or ripping of cell walls. In effect, mechanical damage is but a minor part of the host-parasite relationship. The friction from rubbing of cells as the nematodes crawl or move over them is so slight as to appear inconsequential. Cells collapsed when the contents were digested and consumed, but not from the nemas physically pushing through or between the cells. The nemas might take advantage of inter-cellular spaces, but do not use them exclusively. Mechanical injury as such is a secondary effect resulting from the hyperplastic growth of the pericycle exerting pressure on the surrounding tissues.

As the tumors increased in thickness, the endodermis was pushed outward and ruptured. The cortex was in turn crushed against the exodermis, the intercellular spaces disappeared, and the cells became compressed and distorted beyond recognition (Fig. 4-B). The swelling of the root in the lesion area is mainly due to the growth of the pericycle tumor, and secondarily to the hypertrophy of cortex and tumor cells and deposition of wound gum. In open parts of the lesions where the cortex had been completely destroyed, large lumps of pericycle tumors protruded into the lesion cavity. The diameter of the roots through lesions located 10–30 mm or more from the tip were 0.5–1.5 mm more than the adjacent healthy portions of the same root. The cortex varied from 280 to 400 μ thick in healthy roots and from 164 to 409 μ over lesions without tumors. The ratio of thickness of cortex to tumor in 4 lesions was as follows: 87–409 μ; 102–360 μ; 156–230 μ; 154–256 μ. Pericycle tumors have been observed only in primary roots.

Discussion.—With gnotobiotic cultures it has been possible to study the interaction of burrowing nematodes and citrus roots without the influence of other organisms. The nemas enter into a true antagonistic symbiotic relationship with the citrus roots, and are able in themselves to induce striking as well as profound changes in the characteristics of cells and tissues of the parasitized roots. Mechanical activities of the parasite are not sufficient to account for the nature and extent of the lesions formed; therefore, an explanation must be sought in the physiologic aspects of the relationship.

The process by which burrowing nematodes become established in citrus roots may be divided into 3 fairly distinct phases, comparable to the scheme devised by Brown for establishment of a fungus parasite in a host (2).

1) *The activities that precede penetration of the roots by burrowing nematodes.*—These are: migration of the nemas in search of healthy roots suitable as food; location of the root tip, presumably being guided by organic substances released by the activity growing root tip; and orientation in the root hair zone, with feeding of the parasite on the tender epidermal cells beginning here. The success of the nematodes in accomplishing these events presupposes active rootlet growth, with concomitant production of healthy succulent tissues acceptable to burrowing nematodes.

2) *The actual process of entering the roots.*—Destruction of the epidermis and cortex and formation of the entrance burrows are the second phase of these phenomena.

3) *Those phenomena that occur after the nemas penetrate the root and enter into intimate association with the citrus roots.*—These include activities on the part of the nematodes, responses by the plant to the metabolites released by the nematodes, and possibly plant reaction to toxic substances produced by the plant. Cells and tissues are destroyed by the nematode's digestive and feeding activities. Yet there seems to be no generalized necrosis of cells or putrefactive decay. The parasite does not cause a putrefaction as ordinarily understood; however, the dissolution or

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Fig. 4. *A*) Cross section through disorganized tissue in a gall caused by burrowing nematodes at the tip of a grapefruit root (×310). *B*) Cross section through a grapefruit root containing cavities and tumors caused by burrowing nematodes; a) cavities, b) pericycle tumor, c) displaced endodermis, d) crushed cortex (×70).

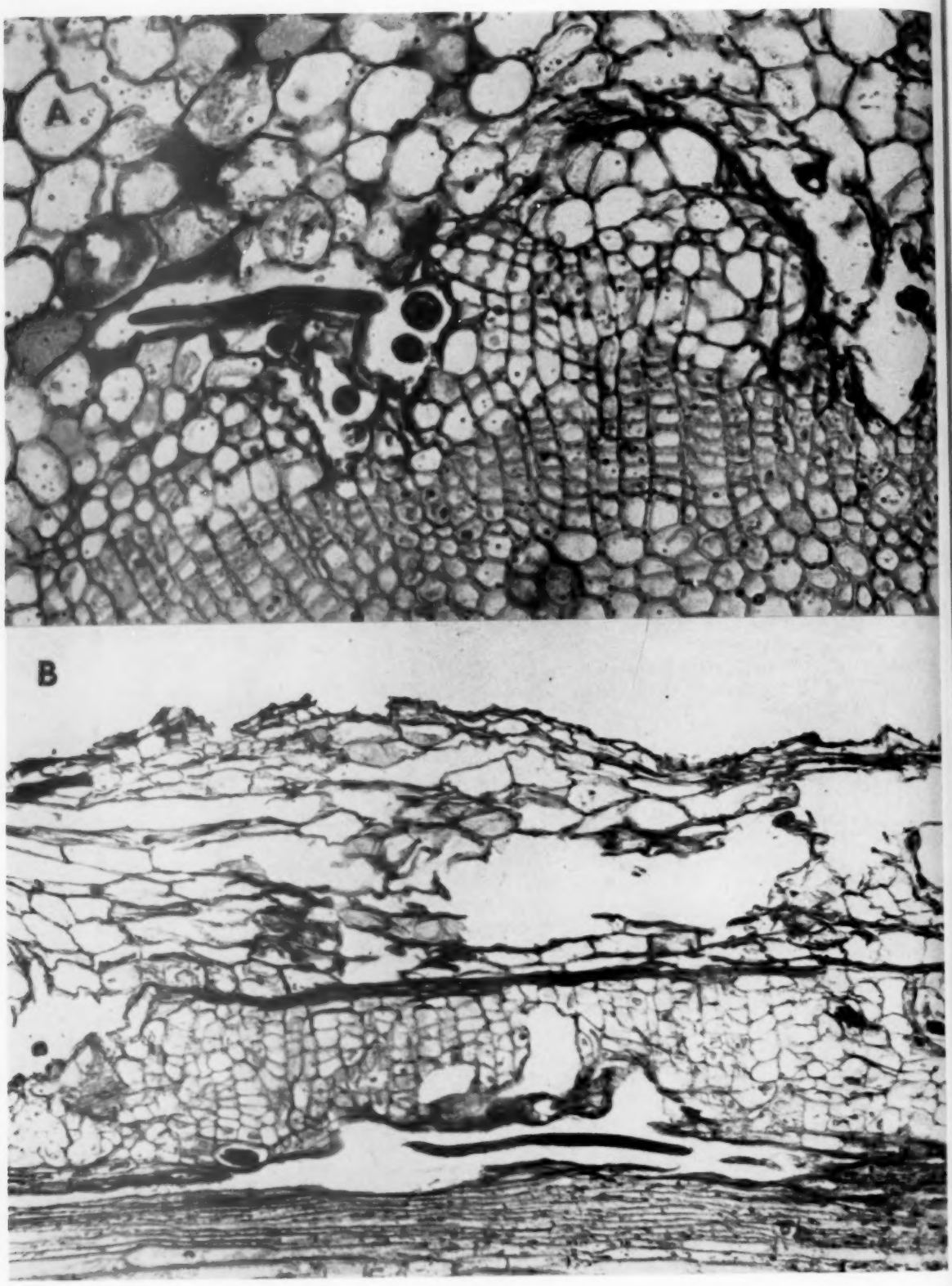

FIG. 5. *A*) Cross section of a grapefruit root in which incipient tumors have started from the pericycle as a result of burrowing nematode activity (×340). *B*) Longitudinal section through a pericycle tumor caused by burrowing nematodes in a grapefruit root (×140).

lysis of the root cells and their contents in the presence of the nematode and/or host metabolites constitutes, in effect, a decay. This lytic process, resulting in the formation of tunnels and cavities in the roots, is most probably accomplished by the action of enzymes secreted by the nematodes. These would include enzymes such as cellulase and pectolytic enzymes able to digest carbohydrates, and proteolytic enzymes that digest the cytoplasm and nuclear materials. These substances would account for the strongly lytic action observed here and also noted by Cobb (4), Goodey (8), and Steiner and Buhrer (10).

Cells and tissues exposed to burrowing nematode activity do not suberize and do not seem to dry, and there is no formation of wound phellogen or wound cork in and about the lesions. The reasons are unknown, but there is a possibility that nematode metabolities or substances inhibit the formation of adventitious wound phellogen. The formation of a layer of wound cork, isolating the nematodes from uninvaded portions of the root, would be inimical to the well-being of the parasites. Preventing the formation of wound cork would assure the parasite of an abundant supply of suitable food.

Growth-stimulating substances, presumably elaborated and released by the nematodes, lead to hyperplasia of the pericycle and root tip, but there is no undue branching of rootlets in this disease, as in some other nematode-caused disorders. Hyperplasia of the pericycle is a phenomenon occurring in the primary roots, that so far has not been observed in fibrous roots of citrus. Pericycle tumor formation, occurring only when burrowing nematodes pass through the endodermis, indicates that the endodermis acts as a barrier that either prevents passage of nemic metabolites into the stele or changes them so that they become innocuous. Whatever substances permeate the endodermis must of necessity pass through the living protoplast and be subject to possible modification. This supposes a filtering effect on the part of the endodermis. The resulting tumors and galls are reactions on the part of the plant, and might be considered a kind of defense mechanism. Conversely, hyperplasia of the pericycle could be a mechanism that assures an abundant supply of plant substances suitable for nematode consumption. This in turn leads to the possibility that the function of these tumors in relation to burrowing nematodes is comparable to the function of giant cells in relation to root-knot nematodes (3). However, it must be kept in mind that *Radopholus similis* is quite mobile even inside the roots, feeding where it wills, in contrast to root-knot nematodes, which are sedentary.

The disappearance of starch in and around lesions induced by burrowing nematodes is reverse to the accumulation of starch noted by Kostoff and Kendall in the region of root-knot galls (9).

In addition to these effects noted, it is possible that other nemic influences exist, such as the release and translocation of metabolites to other parts of the tree, where they may cause disturbances at a distance from the site of nematode activity. It is also not known how long the stimulating effects of burrowing nematodes persist in the root and affect the plant after the nematodes leave a root.

Radopholus similis does not kill the roots, even though there is a reduction in growth of roots. It is conceivable that, if a large enough population of the parasites developed in a lesion, the attacked roots would be completely consumed.

No studies have been made on the influence of burrowing nematodes alone on the well-being of a large citrus tree for a long time. It is unknown whether burrowing nematodes alone could produce the syndrome typical of spreading decline. Such an experiment would require that gnotobiotic association of citrus and burrowing nematodes be maintained for long periods. So far, this has not been possible.

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A PHYTOPATHOLOGICAL NOTE

Transmission of Wheat Streak Mosaic Virus to Corn by the Eriophyid Mite, Aceria tulipae.[1] W. H. Sill, Jr., and Maria S. del Rosario.[2] A strain of wheat streak mosaic virus isolated from naturally infected corn, *Zea mays* L., by Finley,[3] sent to the senior author for study, was reported as a new strain (white mottle) by Lal et al.[4] This strain was used to test whether this virus could be transmitted from wheat, *Triticum vulgare* L., to corn and then back to wheat by the eriophyid mite vector, *Aceria tulipae* (Keifer).[5] Finley[3] did not find the mite in naturally infected sweet corn in the field in Idaho and left some doubt concerning its role as a vector to corn.

In the field in Kansas, *A. tulipae* has been found on corn only rarely by the authors and others making annual surveys. Most field corn varieties grown in significant quantity in the state are either immune or highly resistant to the virus,[6] and no naturally infected corn plants have been seen in the field thus far. This is not surprising, for symptoms in field corn tend to be diffuse and somewhat evanescent.[6, 7] Connin[8] reported several corn varieties susceptible to mite infestation, but did not transmit the virus to corn by the mite.

A. tulipae is found with extreme difficulty in the field on corn because it is so small, 270 μ long. In the greenhouse, mites usually colonize with some difficulty on young corn plants, often in the leaf whorl, and tend to disappear or disperse as the plants mature. This disappearance from older plants was also observed by Connin.[8] It is very easy, therefore, to miss the small and rather infrequent colonies on a small corn plant, and on a large plant the search is usually fruitless. Small corn plants heavily infested with mites in the greenhouse occasionally develop longitudinally rolled and trapped leaves, but these symptoms have not been observed on older infested plants in the greenhouse, or at any time on corn plants in the field. Consequently, this indication of mite infestation probably will not be helpful in locating mite-infested or virus-infected plants in the field, as is quite often the case with wheat.

Table 1.—*Transmission of wheat streak mosaic virus to several corn varieties by the mite vector, Aceria tulipae (Keifer)*

Variety or P.I. no.	No. trials	Total no. plants	Plants developing mite colonies	Mite-infested plants showing virus symptoms
K2234	3	8	7	5
Midland	3	6	6	5
Falconer	2	4	4	2
K1639	4	10	9	4
Golden Giant, Sweet	5	14	7	7

Viruliferous mites, either singly or in colonies, were transferred from virus-infected Pawnee winter wheat plants (C.I. 11669) to several varieties of healthy corn plants chosen because of their systemic susceptibility to the virus by manual abrasive inoculation.[6] Table 1 gives results of the transmission tests. Mite colonies and virus symptoms developed on some plants of all 5 corn varieties. Symptoms were typical of those described for corn by McKinney,[7] Finley,[3] and Sill and Agusiobo.[6] Mites that had colonized on the various corn varieties were moved from infected corn plants to 24 healthy Pawnee wheat plants. Colonies developed on all 24 plants, and virus symptoms on ten.

The experimental evidence supports the view that *A. tulipae* can colonize on corn and return to wheat, and transmit wheat streak mosaic virus to both. Probably the virus is often transmitted to corn in the field, but only a few susceptible sweet corn varieties have thus far developed severe symptoms and been damaged.[3, 6] Even though this disease will probably never be of great economic importance on corn, this host must now be considered another possible oversummering reservoir for, not only the mite vector,[8] but also the virus.—Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, Kansas State College, Manhattan.

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BOOK LISTING

Kamat, M. N. 1958. **Hand-Book of Tropical Crop Diseases.** PraKash Publishing House, Poona, India. 84 p. Price 2.50 rupees.

This volume is a semi-technical handbook prepared for the use of extension plant pathologists and plant-protection officers in tropical Asia. The first section gives general information on plant diseases, their nature, the various types of symptoms encountered, and control procedures. The second section contains a classified list of diseases, their diagnostic symptoms, and methods of controlling them.